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# CARDIAC CONTUSION

## AN EXPERIMENTAL AND PATHOLOGIC STUDY

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Bright and Beck,[1] in a recent review of the literature pertaining to nonpenetrating wounds of the heart, have called attention to the fact that if a person survives the immediate effects of cardiac contusion, the following things may happen: "(1) the symptoms may disappear hours or days after the accident, and the patient get well; (2) the symptoms may persist for years, and they may be accentuated by exercise; (3) the heart may fail hours or days after the accident; (4) the contusion may soften, and rupture may take place." Their conclusions were supported by a review of twenty-three instances of cardiac contusion collected from the literature in which the immediate effects of the cardiac injury were not fatal. Twelve of the patients recovered completely, and eleven died of subsequent heart failure. Beck[2] subsequently published an account of five cases of his own in which cardiac contusion had been survived over periods ranging from six days to sixty-three years. Mönckeberg,[3] in reviewing the subject of traumatic injury of the heart, cited reports of three cases of cardiac contusion with survival for four, six and nine months, respectively, after the injury. These reports included observations at autopsy, which confirmed the diagnosis of cardiac contusion.

In twenty of these thirty-one reported cases of cardiac contusion either there was no record of fractures of ribs or of the sternum, or specific mention was made that fractures were not present. If serious myocardial injury can be sustained in an injury to the chest without fracture of ribs or of the sternum, it is important to know something

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1. Bright, E. F., and Beck, C. S.: Am. Heart J. **10**:293, 1935.
2. Beck, C. S.: J. A. M. A. **104**:109, 1935.
3. Mönckeberg, J.: Herz und Gefässe, in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1924, vol. 2.

of the pathologic anatomic and histologic character of the cardiac lesions, since other objective evidence of injury may not be present. The manner in which these traumatic lesions organize and undergo repair and the means by which they may be distinguished from myocardial infarcts should be determined.

Thorel [4] stated that in cases of cardiac contusion secondary to crushing injury of the chest it is very difficult to identify the traumatic character of the lesion if any considerable time has elapsed since the injury. Külbs [5] studied the myocardial lesions produced experimentally in dogs by cardiac contusion up to the twelfth day after injury and stated that in about 44 per cent of the cases secondary exudative changes in the lesions gave them a character that might be designated as traumatic myocarditis. Bright and Beck, in studying experimental cardiac contusion in twenty-five dogs, summarized the pathologic changes as follows: "We also know that after a contusion has been inflicted upon the myocardium a process of change takes place in the myocardium. This consists of capillary hemorrhage, infiltration with leucocytes, edema, resolution, and finally scar tissue formation."

In order to study the pathologic anatomy of cardiac contusion it was decided to produce traumatic injuries of the heart in dogs. Külbs produced cardiac contusion in dogs by striking the thorax forcibly with a heavy blunt instrument. In this investigation it was felt that opening the thorax under anesthesia and traumatizing the heart directly would be a more humane as well as more accurately controlled procedure.

## EXPERIMENTAL METHOD

Cardiac contusions were produced in thirty-two adult dogs by opening the thorax to the left of the sternum under ether anesthesia and striking the heart one or more forcible blows. A summary of the experiments is shown in table 1. It may be seen that in thirteen animals the heart was struck with a metal dilator. This instrument weighed 68 Gm. and was 22 cm. in length, and the convex striking surface of the instrument was about 1 cm. in diameter. In nineteen of the dogs the heart was struck with a wooden mallet weighing 335 Gm., measuring 31 cm. in length and having a flat striking surface measuring 4.5 cm. in diameter. With few exceptions all blows struck with a given instrument were administered with about the same force.

With the exception of eight dogs that died or were put to death soon after the contusion (one hour or less), the animals were killed at varying intervals after the procedure up to six months. Dogs showing any manifestations of post-operative pain were kept comfortable with morphine.

4. Thorel, C.: Ergebn. d. allg. Path. u. path. Anat. **9**:559, 1903.
5. Külbs, F.: Mitt. a. d. Grenzgeb. d. Med. u. Chir. **19**:4, 1909.

OBSERVATIONS

A wide variety of anatomic lesions was observed, and their extent and locations are shown in figure 1. As indicated in table 1, the extent of the injury was frequently disproportionate to the severity of the trauma. Some hearts showed more extensive injury resulting from a single blow of the mallet than others that received four blows, which, so far as was known, were administered with approximately the same force as was the single blow. Of the hearts injured with the metal dilator, some sustained more myocardial damage from a single blow than did others from thirty blows.

The most commonly observed site of myocardial injury in seventeen dogs was immediately beneath the area of application of force (fig. 1, *1*). With exception of the lesions in three dogs, these local injuries were most extensive immediately beneath the epicardium. They were irregularly wedged shaped, with the apex of the wedge directed toward the endocardium, and they characteristically showed lateral tears into the myocardium on either side. In twelve dogs the local myocardial destruction did not penetrate the entire thickness of the ventricular wall (fig. 1, *2, 3* and *4*), and in five the lacerations extended through the entire thickness of the right (fig. 1, *6*), left (fig. 1, *5*) or both ventricular walls at the site of contusion. Four other transmural lacerations were encountered. Two were through the interventricular septum (fig. 1, *7*) and not in continuity with the local injury. Of the other two, which were not in continuity with the local injury, one was a tear through the right ventricular wall at the pulmonary conus and the other was in the posterior wall of the left ventricle, near the atrioventricular sulcus.

There were three hearts in which there was nonpenetrating local injury beneath the site of application of trauma but not in continuity with the epicardium. In one (fig. 1, *3*) the injury occupied the middle third of the anterior wall of the left ventricle, beneath the seat of trauma, and was separated from both the endocardium and the epicardium by a layer of intact muscle. In two (fig. 1, *4*) the injury was localized to a subendocardial lamella of muscle. In the last two hearts the reverse of the lesion described as being the most commonly occurring one was encountered. The lesion was wedge shaped, and the base of the wedge was applied to the endocardium, the apex being directed toward but not reaching the epicardium.

In fourteen dogs widely disseminated focal areas of myocardial laceration were encountered (fig. 1, *8*). These were distributed throughout the ventricular myocardium, and although they were most numerous in the region of the contusion, they were frequently encountered

TABLE 1.—*Summary of Data from Experiments in Which Cardiac Contusions Were Produced in Dogs*

| Dog | Parietal Pericardium: Opened | Parietal Pericardium: Not Opened | Blows on Heart with Metal Instrument | Blows on Heart with Wooden Mallet | Immediate Functional Effects of Contusion: None | Extrasystoles | Bradycardia | Tachycardia | Ventricular Fibrillation | Asystole | Posttraumatic Survival: Put to Death | Posttraumatic Survival: Died | Pericarditis: None | Pericarditis: Fibrinous: Local | Pericarditis: Fibrinous: General | Pericarditis: Fibrous: Local | Pericarditis: Fibrous: General | Myocardial Injury: None | Myocardial Injury: Local | Myocardial Injury: Rupture of: Left Ventricle | Myocardial Injury: Rupture of: Right Ventricle | Myocardial Injury: Rupture of: Septal | Myocardial Injury: Disseminated |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 74 | + | .. | .. | 3 | .. | .. | .. | + | + | .. | Immediately | ..... | + | .. | .. | .. | .. | .. | + | .. | + | .. | + |
| 72 | + | .. | ? | .. | .. | .. | .. | .. | .. | + | ..... | Immediately | + | .. | .. | .. | .. | .. | + | .. | + | .. | .. |
| 53 | .. | + | .. | 1 | .. | + | .. | .. | .. | .. | Immediately | ..... | + | .. | .. | .. | .. | .. | + | .. | .. | .. | + |
| 42 | .. | + | .. | 1 | .. | .. | .. | .. | + | .. | Immediately | ..... | + | .. | .. | .. | .. | + | .. | .. | .. | .. | + |
| 40 | .. | + | .. | 4 | .. | .. | .. | .. | + | .. | ..... | Immediately | + | .. | .. | .. | .. | .. | + | + | .. | .. | + |
| 39 | .. | + | .. | 2 | .. | .. | .. | .. | + | .. | ..... | Immediately | + | .. | .. | .. | .. | .. | + | + | + | + | + |
| 34 | .. | + | .. | 1 | .. | .. | .. | .. | .. | + | ..... | Immediately | + | .. | .. | .. | .. | .. | + | .. | + | .. | + |
| 27 | .. | + | .. | 1 | .. | .. | .. | .. | .. | + | ..... | At 1 hour | + | .. | .. | .. | .. | .. | + | .. | .. | .. | + |
| 17 | + | .. | 1 | .. | .. | .. | .. | + | .. | .. | At 3 days | ..... | .. | .. | + | .. | .. | .. | + | .. | .. | .. | + |
| 20 | .. | + | .. | 2 | .. | + | .. | .. | .. | .. | At 5 days | ..... | + | .. | .. | .. | .. | + | .. | .. | .. | .. | .. |
| 57 | .. | + | .. | 1 | + | .. | .. | .. | .. | .. | At 7 days | ..... | + | .. | .. | .. | .. | + | .. | .. | .. | .. | .. |
| 12 | + | .. | ? | .. | ? | .. | .. | .. | .. | .. | At 1 month | ..... | .. | .. | .. | .. | + | .. | + | + | .. | .. | + |
| 23 | .. | + | .. | 1 | .. | + | .. | .. | .. | .. | At 2 months | ..... | .. | .. | .. | .. | + | + | .. | .. | .. | .. | .. |
| 90 | + | .. | 12 | .. | + | .. | .. | .. | .. | .. | At 2 months | ..... | + | .. | .. | .. | .. | + | .. | .. | .. | .. | .. |
| 88 | + | .. | 30 | .. | .. | .. | + | .. | .. | .. | At 2 months | ..... | + | .. | .. | .. | .. | + | + | .. | .. | .. | .. |
| 89 | .. | + | 1 | .. | + | .. | .. | .. | .. | .. | At 2 months | ..... | + | .. | .. | .. | .. | + | .. | .. | .. | .. | .. |
| 85 | + | .. | .. | 1 | .. | + | .. | .. | .. | .. | At 2 months | ..... | + | .. | .. | .. | .. | + | .. | .. | .. | .. | .. |
| 61 | + | .. | .. | 4 | .. | .. | + | .. | + | .. | At 2 months | ..... | .. | .. | .. | .. | + | + | .. | .. | .. | .. | .. |
| 65 | .. | + | .. | 4 | + | .. | .. | .. | .. | .. | At 2 months | ..... | + | .. | .. | .. | .. | + | .. | .. | .. | .. | .. |
| 56 | .. | + | .. | 1 | .. | .. | .. | + | .. | .. | At 3 months | ..... | + | .. | .. | .. | .. | + | .. | .. | .. | .. | .. |
| 54 | .. | + | .. | 1 | ? | .. | .. | .. | .. | .. | At 3 months | ..... | + | .. | .. | .. | .. | .. | .. | .. | .. | .. | + |
| 9 | + | .. | 15 | .. | .. | .. | .. | + | .. | .. | At 3 months | ..... | .. | .. | .. | + | .. | .. | + | + | .. | .. | + |
| 36 | .. | + | .. | 2 | + | .. | .. | .. | .. | .. | At 4 months | ..... | + | .. | .. | .. | .. | .. | + | .. | .. | .. | + |
| 33 | .. | + | .. | 1 | .. | .. | .. | + | .. | .. | At 4 months | ..... | + | .. | .. | .. | .. | .. | .. | .. | .. | .. | + |
| 29 | .. | + | .. | 2 | .. | .. | .. | .. | + | .. | At 4 months | ..... | + | .. | .. | .. | .. | .. | .. | .. | .. | .. | + |
| 18 | + | .. | 1 | .. | + | .. | .. | .. | .. | .. | At 4 months | ..... | .. | .. | .. | + | .. | .. | + | .. | .. | .. | .. |
| 15 | + | .. | 30 | .. | + | .. | .. | .. | .. | .. | At 4 months | ..... | .. | .. | .. | + | .. | .. | + | .. | .. | .. | .. |
| 11 | + | .. | ? | .. | .. | .. | .. | + | .. | .. | At 4 months | ..... | .. | .. | .. | .. | + | + | .. | .. | .. | .. | .. |
| 16 | + | .. | ? | 1 | .. | + | .. | .. | .. | .. | At 6 months | ..... | .. | .. | .. | .. | + | .. | + | .. | .. | .. | + |
| 10 | + | .. | 7 | .. | ? | .. | .. | .. | .. | .. | At 6 months | ..... | .. | .. | .. | + | .. | .. | + | .. | .. | + | + |
| 7 | + | .. | 30 | .. | + | .. | .. | .. | .. | .. | At 6 months | ..... | .. | .. | .. | + | .. | .. | + | .. | .. | .. | .. |
| 6 | + | .. | 13 | .. | .. | .. | .. | .. | + | .. | At 6 months | ..... | .. | .. | .. | + | .. | .. | + | .. | .. | .. | .. |

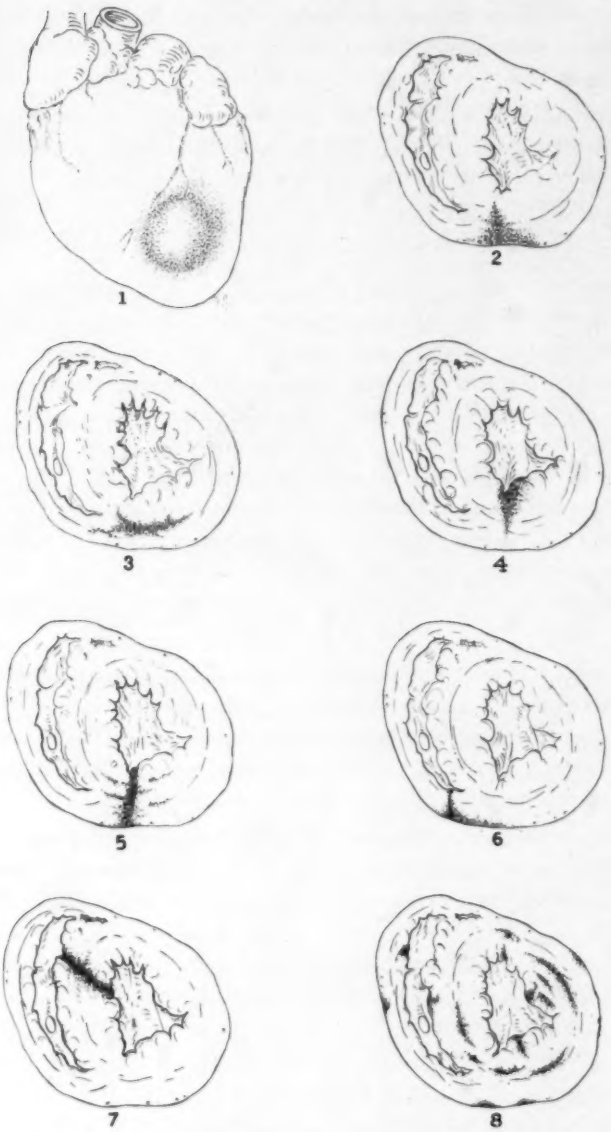


Fig. 1.—Diagrams showing the location and extent of lesions produced by direct contusion of the heart: *1*, site of application of the trauma, showing subepithelial hemorrhage; *2*, local nonpenetrating contusion and laceration (fourteen dogs); *3*, local contusion not in continuity with the site of application of the trauma; the lesion occupies the midportion of the ventricular wall (one dog); *4*, subendocardial laceration beneath the site of contusion (two dogs); *5*, rupture of the left ventricle (four dogs); *6*, rupture of the right ventricle (four dogs); *7*, rupture of the interventricular septum (two dogs); *8*, disseminated myocardial injuries (fourteen dogs).

remotely. In three hearts no myocardial damage was found at the site of direct trauma, but multiple disseminated foci of injury were present elsewhere.

Eleven hearts showed no gross or microscopic evidence of myocardial injury other than of subepicardial or subendocardial petechiae in those that had been recently (within three days) traumatized.

In none of the thirty-two hearts was there auricular laceration or rupture or tearing of valves, chordae tendineae or papillary muscles.

*Relation of Injury to Survival.*—The most severe type of injury was represented by ventricular rupture, and this type of injury was encountered in five of the eight dogs that died soon (within one hour) after trauma. Neither complete ventricular nor interventricular septal laceration was invariably fatal. Two dogs survived injuries which at the end of one and three months, respectively, were represented by scars that extended entirely through the ventricular wall. One dog, killed after six months, had a through and through cicatrix in the posterior apical portion of the interventricular septum. The dogs that survived these severe injuries were not subjected to exercise tolerance tests to determine the degree of functional impairment, but there was no clinical or pathologic evidence of heart failure.

*Relation of Injury to Immediate Functional Changes.*—Eight dogs showed either minor or no changes in pulse rate or in cardiac rhythm following contusion. Subsequent pathologic examination of the hearts of these animals revealed no evidence of myocardial injury in four and scars of local myocardial contusion in four.

In five animals extrasystoles and irregularity of pulse rate developed immediately after contusion. Subsequent pathologic examination revealed no myocardial injury in three and local contusion in two of these dogs.

Bradycardia followed injury in two dogs, one of which showed no myocardial injury, and one, local contusion.

Tachycardia was seen in six dogs after cardiac contusion. Two showed no morphologic evidence of myocardial injury, and three showed widely disseminated focal sites of injury. One in which ventricular fibrillation subsequently developed had a rupture of the wall of the right ventricle.

In seven dogs ventricular fibrillation followed cardiac contusion. In four of these, intracardiac injection of epinephrine [6] or electric

6. In this instance 0.5 cc. of epinephrine hydrochloride (1:20,000) was injected into the left ventricle, and the injection was followed by ventricular massage.

shock[7] was employed to restore normal rhythm. This treatment was successful in three. The permanency of the benefit was not determined in two, owing to the fact that they were put to death as soon as normal rhythm had been restored. In one, the restoration of normal rhythm was permanent so far as a six months' period of observation was concerned.

Ventricular fibrillation appeared to be evidence of more consistently severe myocardial injury than any of the cardiac irregularities just described. Three of the seven dogs showing posttraumatic ventricular fibrillation were found to have complete transmural ventricular laceration; one had widely disseminated focal lacerations of the myocardium, and one, local myocardial contusion. Two showed no morphologic myocardial change. Of the last two, one died after a few minutes of ventricular fibrillation, and one survived two months after a spontaneous reversion to normal rhythm.

Immediate asystole following contusion occurred in three dogs. Two of these had complete laceration of the right ventricle, and there was no recovery from the shock of the contusion. One was successfully resuscitated by an intracardiac injection of epinephrine hydrochloride but died within an hour showing pathologic evidence of severe local and disseminated myocardial injuries.

*Pathologic Studies of Myocardial Injuries.*—The outstanding macroscopic characteristic of the myocardial lesions within twenty-four hours after trauma was hemorrhage, with or without identifiable laceration. Where large defects in ventricular walls had been created, the lacerations were apparent, but the massive interstitial hemorrhage served to mask smaller lacerations. Microscopic examination served to distinguish the tissue disorganization due to crushing from simple myocardial lacerations. At the site of application of trauma, in direct continuity with it, the characteristic lesion was a profound disorganization of tissue with diffuse hemorrhage (fig. 2). Muscle cells were fragmented and displaced, and the orderly streaming of fibers was completely interrupted. In some instances, the local injury was less diffuse and was represented by single or multiple lacerations extending in various directions and to varying depths into the myocardium. Injuries not in direct continuity with the site of contusion consisted in foci of ruptured muscle fibers. Sometimes these ruptures included several and sometimes many adjacent muscle fibers. The extent of the interstitial hemorrhage was invariably greater than that of recognizable myocardial injury. Both large and small lacerations were occupied

7. Broad electrodes were placed against the epicardium, one on each side of the heart, and a 40 volt alternating current was passed through the heart for one second.

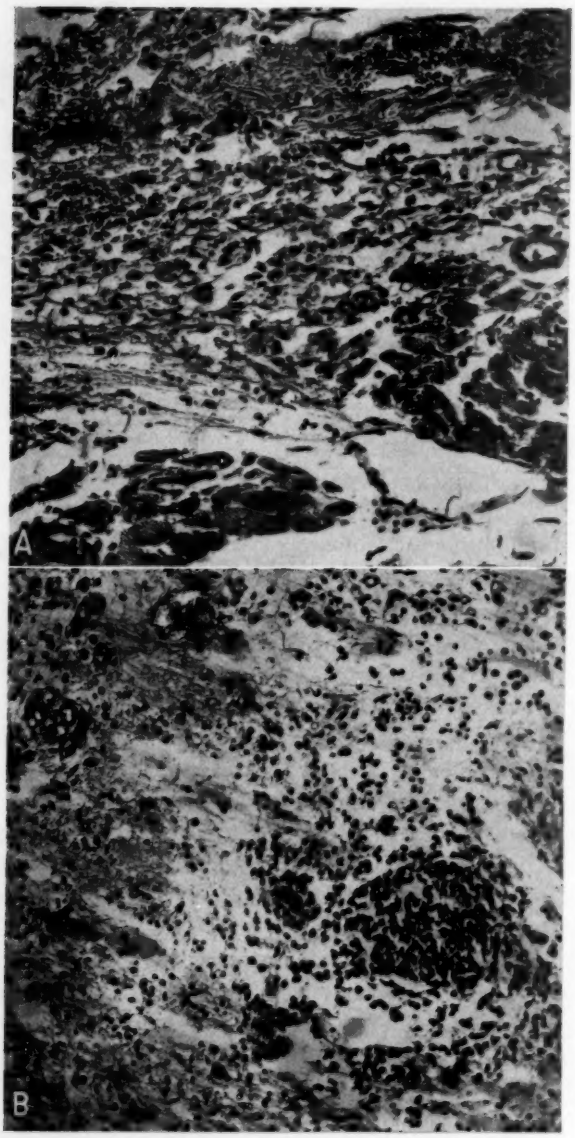


Fig. 2.—*A*, superficial localized myocardial contusion, one hour after injury, showing epicardial and myocardial hemorrhage and edema with disorganization of muscle bundles; × 200. *B*, extensive local myocardial contusion, one week after injury; × 200. Occasional necrotic muscle fibers are seen in a lesion which is edematous and infiltrated with fibrin, mononuclear cells and leukocytes. The hemorrhagic character of the lesion is already less apparent.

by hematomas. As has already been indicated, some of these remote lesions were small, whereas others were complete transmural lacerations of ventricular walls.

Within twenty-four hours the lesions became infiltrated with polymorphonuclear leukocytes, and at the end of three days the leukocytic infiltration was diffuse and in places quite dense. The tissue was edematous, and in addition to the hemorrhage and leukocytes there were lymphocytic infiltration and interstitial skeins of fibrin (fig. 2*B*). Both at the site of contusion and in the remote foci of damage there was obvious necrosis of muscle fibers with loss of nuclear integrity, loss of cross-striations, and swelling and granularity of cytoplasm. Although there was diffuse extravasation of erythrocytes, the hemorrhage appeared less pronounced than in dogs that were examined within twenty-four hours after trauma.

Hearts examined between one and two months after contusion revealed advanced organization of the lesions. Collapse of the damaged tissue had occurred, and where the damage had been superficial there were depressed scars in the epicardium. On incision, these scars were irregularly shaped and sharply defined. They were mottled red and golden brown. Remote, noncommunicating sites of injury did not as a rule lead to epicardial or endocardial deformity because of their deep position in the myocardium. The larger of the remote scattered lesions were so soft that they appeared cystic when incised. Such scars retracted from the cut surface and were red or brown. There were other disseminated lesions throughout the myocardium that were represented by poorly circumscribed, irregularly shaped pale areas, which rarely exceeded 1 mm. in diameter. These were neither elevated nor depressed and were quite opaque.

Microscopically, the larger defects were occupied by a richly vascularized, loose granulation tissue comprised of actively proliferating fibroblasts and many small thick-walled, newly formed blood vessels. The collapse of these scars on incision was apparently due to their rich vascularity. The tissue was diffusely infiltrated by lymphocytes and large mononuclear phagocytes, the latter containing hemosiderin (fig. 3*A*). No hematoidin crystals were seen. Scattered through the scars were isolated groups of muscle cells, some undergoing degeneration, as manifested by karyorrhexis and karyolysis, some atrophic and some well preserved.

The pale gray opaque stippling of the myocardium represented calcification of focally necrotic muscle cells (fig. 3*B*). Single muscle cells or groups of such cells were the seat of coagulation necrosis, and the cytoplasm was occupied by fine and coarse basophilic granules, which were shown to contain calcium by the von Kossa test. Such

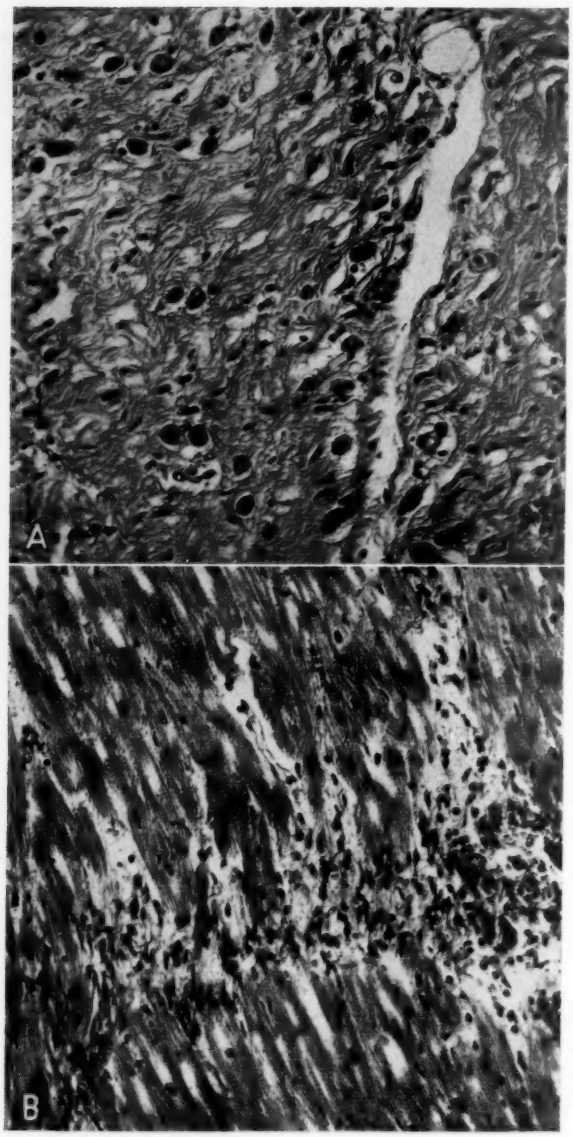


Fig. 3.—*A*, large hemosiderin-containing phagocytes in a large organized myocardial cicatrix six weeks after contusion; × 250. *B*, small linear laceration of the myocardium remote from the site of direct contusion, showing organization; × 200. At one end of the scar calcified necrotic muscle fibers may be seen. The lesion is seen one month after injury.

groups of necrotic muscle cells frequently presented a granulomatous appearance because of the surrounding zone of fibroblasts and mononuclear infiltration.

There were no consistent macroscopic differences in the myocardial lesions in hearts observed three, four or six months after injury. The myocardial scars were uniformly pale and varied in density even in the same heart. After three months, gross hematogenous pigmentation of scars was seen rarely (in two of eleven hearts).

On microscopic examination different scars, even in the same heart, showed marked variation as to density, collagen content, vascularity, fat content and pigmentation. These differences could not be related to the amount of time that had elapsed since the trauma (between three and six months) or to the size or to the location of the scars. Of four hearts having intercellular hemosiderin in scars, two represented three month survival, one four month and one six month survival. In seven hearts examined between three and six months after contusion, no iron-containing pigment was identified with the Pearl reaction despite the fact that all had gross myocardial scars which could be inferred to have been associated with hemorrhage at the time of the original injury. Vascular obliteration by endothelial proliferation was seen in some of the larger scars, particularly those at the sites of contusion. Other scars contained large thin-walled blood vascular spaces, and still others were almost completely avascular. Large scars frequently, and small scars rarely, contained broad interlacing bundles of collagen. In the larger scars hyaline degeneration of fibrous connective tissue was seen focally. Both large and small scars contained varying amounts of adipose tissue, and in some instances the fat predominated over the fibrous connective tissue. In many of the scars, bundles of atrophic muscle were entirely surrounded by connective tissue. No calcification was seen, and the disseminated lesions of focal necrosis that had been observed in hearts examined between one and two months after injury were now represented by noncalcified fibrous scars (fig. 4 *A*). Infiltration by lymphocytes and large mononuclear cells was seen in some (fig. 4 *A*), but for the most part the scars were relatively acellular (fig. 4 *B*).

*Pericardial Changes.*—One month or longer after contusion there were pericardial adhesions in eleven of the twenty-one dogs. In ten of the twenty-one dogs the parietal pericardium had been opened when the heart was traumatized. In nine dogs the pericardium had not been opened at the time of cardiac contusion, and in only one of these were pericardial adhesions found. In this one the pericardial cavity was obliterated by adhesions, but there was no evidence of myocardial injury. It appeared that pericardial adhesions did not result from

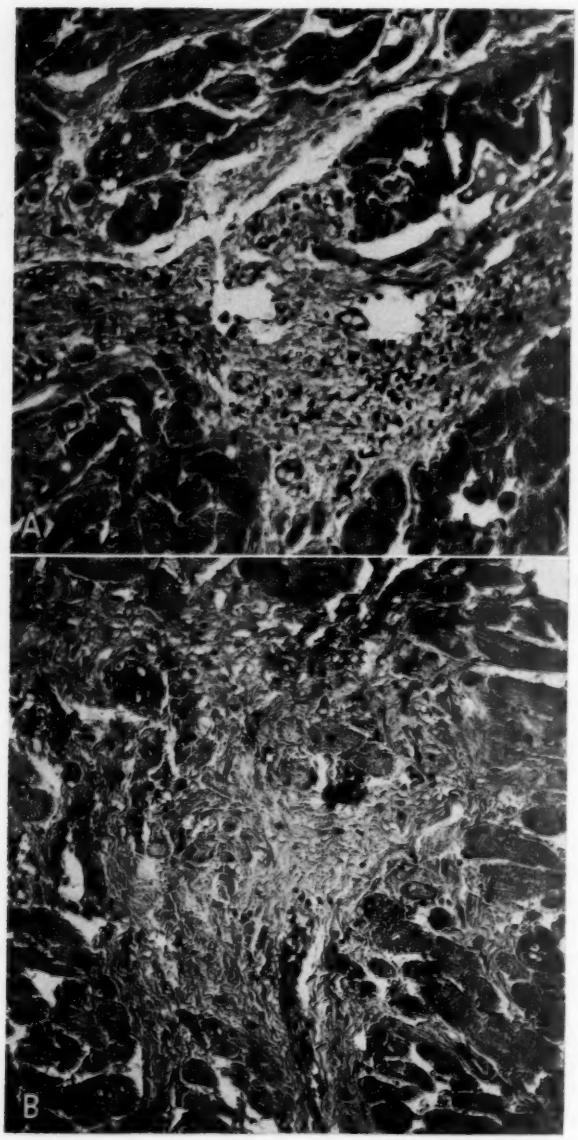


Fig. 4.—*A*, healed focal laceration of the myocardium, showing exudation of lymphocytes three months after contusion; × 250. *B*, healed laceration of the myocardium remote from the site of contusion, six months after injury; × 250.

cardiac contusion but from pericardial incision. In dogs that survived contusion by a month or more there was no recognizable evidence that hemopericardium had ever been present. The densest and longest surviving deposits of hemosiderin were found immediately beneath the epicardium at the site of contusion.

*Endocardial Changes.*—As already indicated, subendocardial, mural and valvular petechiae were seen in hearts recently traumatized (one week or less prior to examination). It appeared likely that in dogs which survived transmural ventricular laceration the site of the laceration was the locus of endocardial thrombosis, but no dogs were killed at an interval of time that made recognition of such a thrombus possible. In hearts examined from three to six months after injury, plaques of nonpigmented endocardial thickening were seen immediately beneath contiguous myocardial scars. These plaques could not, however, be identified as organized mural thrombi.

Many of the myocardial changes seen in this series of dogs with cardiac contusions bore striking resemblances to the gross and microscopic changes seen in myocardial infarction in man. To compare these experimentally produced cardiac contusions with myocardial infarction in man, hearts from ninety-four persons who at the time of death had occlusive disease of the coronary artery were studied. To supplement the human material, hearts from seven dogs in which infarcts had been produced by ligation of coronary arteries were studied (table 2). This material was obtained through the courtesy of Dr. Frederick Mautz, of the department of surgery.

*Comparison of Infarcts and Contusions With Respect to Location and Size.*—There were no absolute differences in the location of lesions either in respect to the chamber affected or in respect to the relation to the endocardium or the epicardium. Three of the eight major experimental infarcts encountered were subepicardial and involved the outer portion of the ventricular wall just as did the majority of lesions due to contusion. In five hearts that were the seat of experimental infarction, disseminated or focal scars were found either near or remote from the major infarct, and in one heart the only infarction recognized was in the form of widely disseminated focal areas of fibrosis. One heart contained an infarct produced experimentally eighteen months earlier which involved the entire thickness of the left ventricle near the apex. This scar was much larger than either of the scars of traumatic ventricular rupture. No statistical study was made of the exact location of the myocardial infarcts in the ninety-four cases of coronary disease in man, but it may be said that there was no constant distribution as regards the chamber affected or the position in relation to the endocardium or epicardium. There

were no significant differences in regard to position or distribution between the experimentally produced myocardial contusions and either the experimentally produced infarcts in dogs or the spontaneous infarcts seen in the human material.

*Comparison of Infarcts and Contusions With Respect to Gross and Microscopic Characteristics.*—In the recently acquired traumatic

TABLE 2.—*Summary of Observations in Dogs' Hearts Showing Infarcts from Experimental Coronary Occlusion*

| Dog | Primary Coronary Ligation | Secondary Coronary Ligation | Survival After Last Coronary Occlusion | Location of Major Infarcts | Location of Disseminated Infarcts |
|---|---|---|---|---|---|
| 121 | Of right coronary, complete | None | 3 days | Entire thickness of wall of right ventricle | None |
| 7 | Of left circumflex, partial | Of left descending, 8 mos. later complete | 1 week | Inner half of anterior wall of left ventricle and interventricular septum | Anterior and lateral wall of left ventricle |
| 48 | Of right coronary, complete | None | 6 weeks | None | Right ventricle |
| 35 | Of left descending, complete | None | 2 mos. | Outer three fourths of anterior wall of left ventricle and interventricular septum | Upper portion of interventricular septum and anterior wall of left ventricle, near base |
| 64 | Of left circumflex, complete | None | 4 mos. | Inner half of posterior wall of left ventricle | Posterior part of interventricular septum and lateral wall of left ventricle |
| 61 | Of left descending, complete | Of left circumflex, 8 mos. later partial | 10 mos. | (1) Middle third of wall of left ventricle, near base<br>(2) Entire thickness of anterior wall of left ventricle, near apex<br>(3) Outer half of posterior wall of left ventricle, near base | Interventricular septum and lateral wall of left ventricle |
| 39 | Of left descending, complete | Of left circumflex, 8 mos. later, partial | 10 mos. | Outer two thirds of anterior wall of left ventricle and interventricular septum | Lateral wall of left ventricle |

injuries of the heart (twenty-four hours or less), laceration and disintegration of tissue were apparent (fig. 2). No such recently acquired experimental infarcts were available for study, but on the basis of the human hearts it is safe to say that they would not have shown such consistently severe structural derangement as was seen in the contusions. It seems likely that with a recently acquired cardiac injury laceration and local structural disintegration of myocardium would as a rule, distinguish such a lesion from a recent infarct. In the human material we studied a spontaneous cardiac rupture at the site of a myocardial infarct, and although it did not show the massive

disintegration of tissue sometimes seen in contusion, there were laceration and widespread interstitial hemorrhage which resembled the traumatic lacerations in dogs very closely.

Hemorrhage was a constant and exceedingly prominent feature of the recently acquired traumatic cardiac lesions, and although there was diffuse extravasation of erythrocytes in recent experimental infarcts (three days and one week old), their number was in no way comparable to that of erythrocytes in the massive hemorrhages of recent cardiac contusion. Karsner and Dwyer [8] described the occurrence of hemorrhage in experimental infarcts in dogs' hearts within thirty minutes after coronary arterial ligation. They observed that the hemorrhage became more pronounced up to forty-eight hours but that at the end of five days the decolorization of erythrocytes tended to obscure this feature of the myocardial lesion. Three recent hemorrhagic infarcts in human hearts were examined, and in none of them was the hemorrhage as prominent as that seen in most of the recent experimental myocardial contusions.

As the lesions caused by contusion organized, the iron was taken up in the form of intracellular hemosiderin, and up to three months these hemosiderin-containing phagocytes were numerous in the larger scars (fig. 3 *A*). The smaller scars were more commonly nonpigmented than pigmented (fig. 4). In none of the experimental infarcts was hemosiderin seen, although in a human myocardial infarct of unknown age occasional hemosiderin-containing phagocytes were found. Karsner and Dwyer [8] noted hemosiderosis in healed experimentally produced myocardial infarcts. The presence, then, of many phagocytes containing iron pigment is probably more likely in lesions of traumatic than in those of ischemic origin but does not constitute an absolute differential feature. The absence of hemosiderin in scars three months or more old is of no significance in excluding contusion inasmuch as hemosiderin was found in only four of eleven traumatic myocardial lesions three months or more after contusion.

In one traumatized heart, disseminated focal areas of myocardial necrosis with calcification were observed one month after contusion (fig. B *B*). No comparable lesions were found in any of the ninety-four cases of human myocardial infarction studied. Identically the same type of focal myocardial calcification was observed, however, in one dog fifty-seven days after ligation of the descending ramus of the left coronary artery. Focal calcification, then, was not pathognomonic of cardiac contusion in dogs.

The myocardial reaction to traumatic injury was relatively uniform at any given stage in the process of repair. Evidence of coexistent

8. Karsner, H. T., and Dwyer, J. E.: J. M. Research **34**:21, 1916.

old and recent injury was not seen to any great degree, as the major injury was inflicted on an otherwise normal heart, was inflicted instantaneously and was not succeeded by subsequent injuries. The lesions following contusion were, however, not entirely static. Exudation persisted in some scars as long as three months after contusion (fig. 4*A*). After one week, the exudative cells were principally mononuclear rather than polymorphonuclear, but they provided evidence that an exudative response to traumatic injury could and did persist in an otherwise extensively cicatrized lesion. Progressive degeneration and atrophy of muscle fibers incorporated in scar tissue were seen up to three months after myocardial contusion. In none of the experimentally traumatized hearts were extensive healed and recent injuries seen concomitantly. This difference between traumatic and ischemic myocardial injury was not seen in four of the dogs having experimentally produced myocardial infarction because they too sustained their major injury at one time, i. e., immediately following the coronary ligation. In three dogs, however, in which coronary ligation was carried out in two successive stages, evidence of separate injuries of different ages was discernible, and in them there was coexistence of lesions indicating old and recent injury. This coexistence of old and recent damage served to distinguish these three hearts from any of the hearts with myocardial contusion.

The finding of coexistent old and recent lesions in human hearts the seat of infarction is very common. In 86 per cent of the ninety-four human hearts with occlusive coronary disease Moritz and Beck[9] reported that there was postmortem evidence of repeated episodes of myocardial injury due to ischemia. It may be said then that an important distinguishing feature between lesions of myocardial contusion and those of myocardial ischemia is that in the former the myocardial changes are relatively uniform, whereas in the latter there is more apt to be evidence of major injuries of varying age. The question immediately arises as to how to recognize a contusion in a heart previously damaged by ischemia. It is entirely possible for a patient with severe coronary disease to sustain myocardial contusion. If the patient survives the immediate effects of the contusion, myocardial lesions of different ages, representing two or more severe injuries, may be encountered. The following case is cited in illustration of this point.

C. P., a white man 74 years of age, was admitted to the emergency ward with multiple lacerations and contusions of the face and scalp, fractures of the third and fourth ribs at the costochondral junctions on the right side and palpable ruptures of the internal and external lateral ligaments of the left knee. He had

9. Moritz, A. R., and Beck, C. S.: Am. Heart J. **10**:874, 1935.

sustained these injuries as the result of being struck by an automobile. He was admitted almost immediately after the accident, was excited and resisted treatment vigorously. For the first two hours the blood pressure was maintained at 150 systolic and 100 diastolic. Two and a half hours after admission and approximately three hours after the accident, the blood pressure had fallen to 70 systolic and 50 diastolic, and the patient was treated for shock. After 500 cc. of dextrose had been administered intravenously, the blood pressure rose to 110 systolic and 70 diastolic, and in the course of the next twenty-four hours, fell again, but rose to its original level following digitalization. A pulse deficit developed, and there was auricular fibrillation, as well as increasing pulmonary edema, despite the maintenance of normal blood pressure and the digitalization. The heart failure progressed in severity, and the patient died on the tenth day following injury.

The postmortem diagnoses were: cardiac contusion with hemorrhagic focal necrosis at the site of a healed infarct of the right ventricle; severe coronary arteriosclerosis without recent thrombosis; fractures of the third and fourth ribs on the right; acute fibrinous pericarditis; cardiac hypertrophy and dilatation (670 Gm.); arterial nephrosclerosis; chronic peptic ulcer of the duodenum; multiple interstitial pulmonary hemorrhages; fractures of the left fibula; laceration of the ligaments of the left knee; abrasions of the face and of the lower extremities; chronic cholecystitis; arteriosclerosis; pulmonary emphysema.

Microscopic examination showed laceration of the entire thickness of the wall of the right ventricle at the site of an aneurysmal protuberance, which measured 3 cm. in diameter and was elevated 1 cm. above the surrounding epicardium. The actual laceration was relatively small, and the attenuated muscle fiber which partially covered the defect was the seat of coagulation necrosis. The protuberance itself was occupied by an organizing hematoma, which communicated with the right ventricle. The adjacent degenerate myocardium was diffusely hemorrhagic and densely infiltrated by leukocytes. The acute degenerative and inflammatory changes had taken place in myocardium that was extensively replaced by dense fibrous connective tissue. This scar tissue was richly vascularized and had replaced about half of the thickness of the muscle. There were multiple areas of coagulation necrosis, some associated with interstitial hemorrhage, in various portions of the walls of the right and left ventricles and in the interventricular septum.

There was a large area of subendocardial hemorrhage in the right auricle, and there was diffuse infiltration of lymphocytes throughout the adjacent myocardium.

In the case just described, the definite evidence of a crushing injury to the chest, with fracture of several precordial ribs, together with the absence of any recent coronary thrombosis, made the diagnosis of cardiac contusion tenable. If this man had survived the accident for a longer period, and if the objective evidence of thoracic injury in the form of fractured ribs had not been present, the problem would have been far more difficult to solve.

The most obvious means of distinguishing between cardiac contusion and infarction objectively would be by the identification of coronary occlusion estimated to be of about the same age as the myocardial lesion in question. That this is not always feasible is indicated by a study of twenty human hearts in which there were large cicatrized

infarcts and in which a careful examination of both coronary arteries and their major branches was made. In only nine of the twenty hearts could the site of remote coronary occlusion be identified. It is rare, however, to find a recent myocardial infarct without a corresponding coronary arterial occlusion. With an acute myocardial lesion the presence or absence of coronary occlusion is significant in determining whether the lesion is due to ischemia or not. With a myocardial scar, however, the absence of demonstrable coronary occlusion does not contribute any information as to the cause of the scar and does not exclude the possibility of the scar having been caused by infarction.

SUMMARY

The objective pathologic criteria for distinguishing between a cardiac contusion and a cardiac infarct vary in usefulness according to the age of the lesion. In the case of a recent myocardial lesion, the only evidence that should almost invariably serve to identify an otherwise indeterminate injury as an infarct is the finding of recent coronary occlusion. Pathologic changes more likely to be found in early contusion than in early infarction include massive interstitial hemorrhage, laceration and tissue disorganization. Since all of these changes may be seen following spontaneous rupture of an early infarct, they are not conclusive. In the case of an older myocardial lesion there is no means of distinguishing objectively between contusion and infarction. Deposits of hemosiderin in myocardial scars are more likely to be seen in healed contusions than in healed infarcts, but since hemosiderin is seen occasionally in healed infarcts its presence is not conclusive. Three months after injury hemosiderin is found infrequently in traumatic scars, so that its absence in no way excludes the possibility of a lesion having been of traumatic origin. The presence or absence of remote coronary occlusion does not serve to identify a myocardial scar as having resulted from infarction or contusion inasmuch as a heart the seat of occlusive coronary disease may have a superimposed traumatic lesion and a heart with a large healed infarct may have no demonstrable coronary occlusion. The pathologic characteristics of the scars of myocardial contusion and infarction are frequently identical, and the presumptive nature of their origin must be determined by historical data rather than by postmortem examination.

# TYPING OF BLOOD AND SEMINAL STAINS BY MEANS OF THE ABSORPTION TEST

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In many of the deaths investigated by the office of the chief medical examiner of the city of New York during the past two and a half years the possibilities of the application of serologic methods for the typing of dried blood stains were evident. The use of these tests has been described by several reliable European and American workers, who do not hesitate to recommend them as a means of furnishing additional evidence in criminal cases. Gettler and Kramer [1] recently published a paper entitled "Blood Grouping in Forensic Medicine" in which they mentioned that their results with the grouping of dried and old blood stains were disappointing. Their report as to the practical value and absolute reliability of the tests contradicts the conclusions of other workers. The discrepancy between their results and those of other laboratories and also the obvious usefulness of a reliable and consistent method for the typing of dried blood and dried bloody fluids suggested a reexamination of the problem.

In reviewing the literature it is found that there is not any unanimity of opinion among forensic experts concerning the most efficient method of typing dried materials. Lattes [2] in testing for the group of dried bloody material utilized the agglutinins in the dried serum, but unfortunately the excellent results which he reports were not duplicated with the same consistent success by workers in other forensic institutes. Thereafter Holzer [3] applied the principles of the absorption test in his technic and devised a method which has been widely accepted as an improved method for the determination of the blood group of dried materials. In contradistinction to Lattes' use of the agglutinin test Holzer makes use of the agglutinogens present in dried blood and other material, in determining group specificity. Favorable reports have been forthcoming from other workers who have used the absorption test (Strassmann [4]); the absorption technic has been

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1. Gettler, A. O., and Kramer, H. E.: J. Immunol. **31**:578, 1936.

2. Lattes, L.: (*a*) Individuality of the Blood in Biology and in Clinical and Forensic Medicine, New York, Oxford University Press, 1932; (*b*) Arch. f. Kriminol. **99**:102 and 201, 1936.

3. Holzer, F. J.: Deutsche Ztschr. f. d. ges. gerichtl. Med. **16**:445, 1931.

4. Strassmann, G.: Deutsche Ztschr. f. d. ges. gerichtl. Med. **19**:302, 1932; **23**:186, 1934.

applied rather generally in the German forensic institutes. Merkel,[5] for instance, utilizing this technic in the institute in Munich, has reported certain difficulties in its application. Richter-Heimbach,[6] working under Merkel's direction in her doctor's thesis concluded that there was a satisfactory degree of probability favoring the correctness of the results. However, she strongly advised that the greatest caution should be observed in the use of the absorption method in criminal cases, thus agreeing with Schiff.[7] It must be borne in mind that the ideal conditions of a laboratory experiment are rarely if ever furnished in a forensic case; in the latter instance nothing may be known concerning atmospheric or other deleterious influences or the length of time for which the material in question has been exposed before examination. This difference between the conditions of laboratory experiments and those encountered in forensic cases probably explains the divergence in opinion concerning the significance of the results and their reliability as set down in two recent publications. Therkelsen,[8] who conducted a detailed study at the Forensic Institute in Copenhagen, concluded that at present it is possible to determine correctly the blood group of dried bloody material and also the group specificity of other body secretions, especially that of semen. Hirszfeld[9] and his former assistant, Lewinsky,[10] are of the contrary opinion. American workers, namely, Wiener[11] and Levine,[12] who mentioned the question briefly, and also certain German workers, for example, Ponsold,[13] described the method and gave its practical application, pointing out the errors which are liable to arise. Ratcliffe,[14] attempting to determine the "cell group identification of dried blood spots and traces of saliva," reported satisfactory results with the absorption method. Hirszfeld and Lewinsky are the only authors who have been emphatic in their doubt as to the possibility of accurately and consistently determining the group to which dried blood or seminal stains belong. These workers emphasized that any article of clothing which has been worn for even a short time may contain the group-specific substances of the owner, which are excreted in the sweat and other fluid excretions. Such agglutinogens on clothing may occur in places where suspicious stains are found. The combination of agglutinogens derived from the subject's secretions and from the stain in question may readily give confusing results. This objection cannot be entirely refuted. It is only when unstained portions of the clothing of the person under investigation do not show the presence of any agglutinogens that the determination based on a dried stain has practical significance. In this connection it may be pointed out that in one case in which the method was used to type a blood stain on a cushion of the front seat of a motor car the results were vitiated because the examination of the fabric of the seat yielded various agglutinogens in

5. Merkel, H., in Festschrift Heinrich Zangger, Zurich, Rascher, 1935, vol. 1, p. 120.

6. Richter-Heimbach, H.: Blutgruppenbestimmung an Blutflecken, Inaug. Dissert., Munich, 1934.

7. Schiff, F.: Die Technik der Blutgruppenuntersuchung, Berlin, Julius Springer, 1932.

8. Therkelsen, F.: Ztschr. f. Rassenphysiol. **8**:98, 1936; **9**:1, 1937.

9. Hirszfeld, L.: Deutsche Ztschr. f. d. ges. gerichtl. Med. **27**:189, 1936.

10. Lewinsky, W.: Deutsche Ztschr. f. d. ges. gerichtl. Med. **27**:194, 1936.

11. Wiener, A.: Blood Groups and Blood Transfusions, Springfield, Ill., Charles C. Thomas, Publisher, 1935.

12. Levine, P.: J. Lab. & Clin. Med. **20**:785, 1935.

13. Ponsold, A.: Deutsche Ztschr. f. d. ges. gerichtl. Med. **23**:46, 1934.

14. Ratcliffe, A. W.: J. Lab. & Clin. Med. **22**:191, 1936.

different tested areas which did not show any obvious stains, presumably owing to nonspecific substances capable of absorbing antibodies. The papers of Hirszfeld and Lewinsky indicate sufficiently the difficulties in the solution of the problem. But even these authors described a few cases in which successful determinations of the groups of dried stained material were carried out. In spite of these, sometimes valid, objections to the test, it seemed justified to reexamine the reliability of testing stains by the absorption method. Even an occasional successful application would warrant trial of this method. The paper of Boyd and Boyd[15] reporting 100 per cent successful retyping of dried blood spots strongly supports the use of the method in practical cases.

Thus far only the four classic groups of Landsteiner, namely, O, A, B and AB, have been the subjects of study. In spite of Therkelsen's investigations the determination of subgroups $A_1$ and $A_2$ and of the types M, N, MN and P in dried material has not been sufficiently developed to allow its practical application. One must bear in mind that a biologic test such as is employed in the determination of the group of a dried blood stain should be clearcut and consistently reliable. Partly because of this strict requirement such tests have had a relatively limited application in the courts.

In reexamining the problem as to the validity and consistency of the absorption test it seemed advisable to determine first which method of the numerous ones employing the absorption principle yielded the best results. In this connection the following features may be emphasized:

1. The centrifuge method is far superior to the porcelain plate method. With the former the grading of the amount of agglutination is readily carried out macroscopically, the consistency of the results having been shown by numerous microscopic control readings.

2. Although some workers, namely, Merkel, Holzer and Strassmann, have employed group O serum, containing agglutinins $\alpha$ and $\beta$, Schiff, Wiener and recently Boyd and Boyd have recommended that the absorption test be performed either with a mixture of A and B serums ($\beta$ and $\alpha$ agglutinins) or with separate A and B serums. It has not been possible to establish any advantage of one method over the other in this series. The higher incidence of persons who are of blood group O facilitates a supply of O serum with equally high titer of agglutinins $\alpha$ and $\beta$. Both methods have been tried out, often simultaneously, one method serving as a control for the other.

3. The selection of potent absorbing serum has been found to be very important. In order to obtain serum of sufficiently high titer in sufficient amounts, different specimens of the same group are pooled and then titrated. Only serum having a titer of at least 96 or, if possible, 192 should be used. For test cells, sensitive group A and group B erythrocytes in a 5 per cent suspension in physiologic solution of sodium chloride are to be used. Fresh red cells should be used, or fresh saline suspensions of cells made from a blood clot kept in the refrigerator

15. Boyd, W. C., and Boyd, L. G.: J. Immunol. **33**:159, 1937.

under sterile conditions. It has been found that cell suspensions older than two days, although grossly and microscopically unchanged, show a marked decrease in their sensitivity to agglutination. For example the same test serum which reacted with old red cell suspensions to a low titer reacted with fresh red cell suspensions to a high titer.

4. In carrying out the absorption test the tubes should be allowed to remain at room temperature (from 17 to 20 C.) for two hours and then over night at icebox temperature (about plus 4 C.). In many cases the absorption may be complete in from one to two hours, although not infrequently the process requires more time. It has been found that with longer absorption—extending over several days at icebox temperature—the results are not different from those obtained with the routine period of twenty-four hours.

For more detailed information as to the performance of the absorption test, the reader is referred to the articles by Wiener,[11] Schiff[7] and the Boyds.[15] The tests reported in this paper were carried out according to the principles stated here and following the suggestions of the two first-named authors.

## EXPERIMENTAL METHOD

The material used for the tests consisted of samples of blood obtained through the Department of Laboratories, Bellevue Hospital, also of blood obtained at autopsies performed by the Office of the Chief Medical Examiner of the City of New York and also fresh blood from students of New York University College of Medicine and technicians and physicians of Bellevue Hospital. Preparations were made by soaking filter paper, linen and cotton cloth with blood and then drying them. Dried seminal stains were prepared for testing by removing the contents of seminal vesicles at the autopsy table. Seminal material was taken only from corpses in which there was no obvious infection of the genitalia and also no generalized septic infection. The seminal vesicles were dissected carefully, and the mucoid grayish yellow fluid, free from blood, was collected in small tubes. The presence of spermatozoa was determined by microscopic examination of a smear stained with gentian violet, after which the fluid, diluted with physiologic solution of sodium chloride, was smeared on filter paper, linen or cotton and dried. The drying was allowed to take place at room temperature. The blood of the cadaver from which semen was thus obtained was also typed. The dried specimens were then examined by the same process as were the dried blood stains, after variable periods of time had elapsed, namely, from three days up to more than one year. Although the objection may be made that the seminal vesicular fluid is not of the same composition as that of semen obtained from ejaculation, the method employed to obtain seminal stains was chosen for the reason that only thus was it possible to collect the necessary number of specimens of the four different groups. The specimens obtained contained large numbers of spermatozoa and also some, if not all, of the ingredients of ejaculated seminal fluid. This seemed to indicate that the experimental conditions were similar to those of Landsteiner and Levine,[16] who tried out their tests on fresh condom specimens.

16. Landsteiner, K., and Levine, P.: J. Immunol. **12**:415, 1926.

The dried samples of blood and semen were stored in small boxes, both cardboard and metal, at room temperature, and covered to protect them from sunlight. Although the humidity during the summer months was frequently very high, putrefaction was not noticeable in the dried stains. In view of the unfavorable results of Gettler and Kramer on decomposed material, it should be emphasized that such material was not employed in the present series, so that an evaluation of the amount of destructive effect of putrefaction or of sunlight on the agglutinogens does not enter into the consideration here.

As has been observed in other laboratories, the period of time elapsing between the obtaining of the blood and the time of the absorption test was not of any great significance in the outcome of the experiments. The same results were obtained

TABLE 1.—*Protocol of an Absorption Test with Controls*

| Description | Material | Test Serum | Test Cells | Agglutination in Progressive Serum Dilutions* 1:6 | 1:12 | 1:24 | 1:48 | 1:96 | 1:192 | Titer | Group |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Determination of original titer of test serum | Known serum plus known cells | A | B | 3 | 3 | 2 | 2 | 1 | ± | 96 | |
| | | B | A | 3 | 3 | 2½ | 2 | 1 | 0 | 96 | |
| Control reactions to determine agglutinogen absorption with known groups | Bloody material of group O + test serum + test cells | A | B | 3 | 3 | 2½ | 2½ | 1½ | ± | 96 | O |
| | | B | A | 3 | 2½ | 2½ | 2 | 1 | 0 | 96 | |
| | Bloody material of group A + test serum + test cells | A | B | 3 | 2½ | 1½ | 1 | 1 | 0 | 96 | A |
| | | B | A | 2 | 1 | 0 | | | | 12 | |
| | Bloody material of group B + test serum + test cells | A | B | 2 | 1 | 0 | | | | 12 | B |
| | | B | A | 3 | 2½ | 2 | 1½ | 1 | 0 | 96 | |
| | Bloody material of group AB + test serum + test cells | A | B | 1½ | 1 | 0 | | | | 12 | AB |
| | | B | A | 1 | 0 | | | | | 6 | |
| Nonspecific control reaction | Unstained substratum + test serum + test cells | A | B | 3 | 3 | 3 | 2 | 1 | 0 | 96 | |
| | | B | A | 3 | 3 | 2½ | 2 | 1 | 0 | 96 | |
| Test with unknown stain | Unknown bloody material + test serum + test cells | A | B | 1 | 0 | | | | | 6 | B |
| | | B | A | 3 | 2½ | 2 | 1½ | 1 | 0 | 96 | |

* The degree of agglutination is expressed as 3, 2½, 2, 1½, 1 and ±.

when the tests were carried out only a few days after the preparation of the blood stain as were obtained when the tests were performed six, twelve or eighteen months after their preparation. In those specimens in which difficulty was met and indefinite results obtained, such indecisive results were encountered as frequently following short intervals as following long intervals after the drying. Because of this it seemed unnecessary to subdivide the results obtained according to the age of the material.

Before presenting the results of the present series of tests a sample protocol is shown in table 1, dealing with the determination in an actual case. In the first column the original titration and the reactions of the different serums with known and unknown material are indicated. The second column lists the various materials which entered into the tests. In this column the substances used for the nonspecific control reaction and for the test on the unknown material are shown as the last two items. In performing the tests 20 mg. of the known and 20 mg. of the unknown stained material were used and mixed in test tubes with 0.2 cc. of test

serum diluted 1:2 in physiologic solution of sodium chloride, respectively. The absorptions were allowed to take place for two hours at room temperature and for twenty additional hours at icebox temperature, after which the tubes were centrifugated for from one to two minutes. Progressive dilutions were then made of the supernatant liquid as indicated in columns 5 to 10, the dilutions varying from 1:6 to 1:192. To each of the diluted preparations 1 drop of a 5 per cent suspension of the test cells in saline solution was added, the mixtures were shaken, allowed to stand for ten minutes and then centrifugated for two minutes. The degrees of agglutination as read after a gentle shaking of the centrifuge tubes are given in columns 5 to 10 of table 1. In column 11 the final titer is indicated for each of the tests, and in the last column the group to which the material belongs as determined by the absorption test is indicated. The degree of agglutination is expressed quantitatively by the numerals, 3, 2½, 2, 1½, 1 and ±.

## OBSERVATIONS

The results obtained by testing blood-stained material are presented in table 2. The results obtained with material from the seminal vesicles are indicated in table 3. A summary of the results obtained with bloody and seminal stained material is given in table 4.

It will be noted in tables 2 and 3 that approximately the same percentage of errors occurred for each of the groups whether bloody or seminal material had been tested. Because of the similarity of the results obtained it seems appropriate to discuss the experiments together.

In the series of cases which are being reported in this paper it was not possible to obtain with the samples employed the correct types of a certain small number of bloody and seminal materials; 202 different specimens were examined, and the determinations as to 11 (5.5 per cent) were erroneous. The material at hand is too small for a valid statistical evaluation of any variation in the percentage of errors in the different groups, which applies also to the precentages listed in columns 5 and 11 of tables 2 and 3. Perhaps the percentages in the summaries have more significance, since there are more instances included. In columns 6, 7 and 8 the numbers of determinations (total, positive and negative) are given. It is evident from these columns that more than a single test has been performed on a sample. The apparent large number of negative results represent simply repetition of first tests in which results were negative.

The results listed in the last three columns of tables 2, 3 and 4 indicate the difficulties of the absorption technic as applied in this series. In 25 of 202 tests the first result obtained with the absorption technic was wrong. In some of these cases a subsequent test permitted the detection and correction of the initial error. In 191 of the 202 cases a consistently correct result was obtained. In only 11 of the cases were the results consistently wrong. A short survey of these erroneous cases is given in table 5.

TABLE 2.—*Bloody Material*

| Group | Samples Used | Positive Results | Erroneous Groupings: Total | Erroneous Groupings: Percentage | Determinations: Total | Determinations: Positive | Determinations: Negative | Positive Results with First Test | Negative Results with First Test: Total | Negative Results with First Test: Percentage |
|---|---|---|---|---|---|---|---|---|---|---|
| O | 39 | 37 | 2 | 5 | 99 | 88 | 11 | 36 | 3 | 7 |
| A | 44 | 40 | 4 | 9 | 134 | 100 | 34 | 37 | 7 | 16 |
| B | 17 | 17 | .. | .. | 62 | 57 | 5 | 14 | 3 | 18 |
| AB | 9 | 9 | .. | .. | 32 | 25 | 7 | 8 | 1 | 11 |
| Total | 109 | 103 | 6 | 5.5 | 327 | 270 | 57 | 95 | 14 | 13 |

TABLE 3.—*Material Stained with Semen from the Seminal Vesicles*

| Group | Samples Used | Positive Results | Erroneous Groupings: Total | Erroneous Groupings: Percentage | Determinations: Total | Determinations: Positive | Determinations: Negative | Positive Results with First Test | Negative Results with First Test: Total | Negative Results with First Test: Percentage |
|---|---|---|---|---|---|---|---|---|---|---|
| O | 48 | 47 | 1 | 2 | 80 | 73 | 7 | 44 | 4 | 14 |
| A | 28 | 26 | 2 | 7 | 56 | 39 | 17 | 24 | 4 | 14 |
| B | 14 | 12 | 2 | 14 | 30 | 24 | 6 | 11 | 3 | 21 |
| AB | 3 | 3 | .. | .. | 9 | 4 | 5 | 3 | .. | .. |
| Total | 93 | 88 | 5 | 6.4 | 175 | 140 | 35 | 82 | 11 | 12 |

TABLE 4.—*Summary of Tables 2 and 3 (Bloody and Seminal Material)*

| Group | Samples Used | Positive Results | Erroneous Groupings: Total | Erroneous Groupings: Percentage | Determinations: Total | Determinations: Positive | Determinations: Negative | Positive Results with First Test | Negative Results with First Test: Total | Negative Results with First Test: Percentage |
|---|---|---|---|---|---|---|---|---|---|---|
| O | 87 | 84 | 3 | 3.4 | 179 | 161 | 18 | 80 | 7 | 10 |
| A | 72 | 66 | 6 | 8 | 190 | 139 | 51 | 61 | 11 | 15 |
| B | 31 | 29 | 2 | 7 | 92 | 81 | 11 | 25 | 6 | 19 |
| AB | 12 | 12 | .. | .. | 41 | 29 | 12 | 11 | 1 | 8 |
| Total | 202 | 191 | 11 | 5.5 | 502 | 410 | 92 | 177 | 25 | 13 |

TABLE 5.—*Summary of Erroneous Results*

| Description of Material | Group | Cases | Erroneous Determination |
|---|---|---|---|
| Bloody | O | 2 | No. 51 determined as A |
| | | | No. 83 determined as B |
| | A | 4 | No. 44 determined as O and AB |
| | | | No. 55 determined as O |
| | | | No. 72 determined as O and AB |
| | | | No. 77 determined as B |
| Seminal | O | 1 | No. 65 determined as A |
| | A | 2 | No. 46 determined as O, AB and B (once as A) |
| | | | No. 71 determined as O |
| | B | 2 | No. 39 determined as O |
| | | | No. 41 determined as A and O |

The question now arises as to the explanation of these erroneous results. As to the technic it must be stated, especially in view of the experiences of Boyd and Boyd,[15] that it would have been desirable to repeat the experiments with smaller quantities of the material whenever an agglutinogen was found which did not belong there and to apply, likewise, higher dilutions of a smaller quantity of the serum for the absorbing procedure. How far certain changes may have occurred in the dried material itself, which may have been partly responsible for the errors, is open to discussion. It is known that agglutinins are susceptible to various external influences, such as radiation, heat, humidity and postmortem decomposition. On the other hand, the agglutinogens are relatively more resistant, and this is practically demonstrated by the more consistently satisfactory results obtained with the absorption test, which utilizes the agglutinogens, than with the Lattes test for agglutinin content.

Nevertheless, the agglutinogens are not absolutely resistant to changes by the external factors mentioned. Certain individual qualities of the blood, peculiar to the specimen in question, may play a role in the outcome of the absorption test. For example, certain red cells may contain agglutinogens which are relatively insensitive, and when deterioration occurs, such less sensitive agglutinogens will deteriorate more readily than highly sensitive and abundant agglutinogens. This possibility also applies to agglutinogens present in tissues or cells other than blood. A decrease in the amount or sensitivity of the agglutinogens may be the reason for the erroneous finding of group O in cases in which the material actually belongs to group A, B or AB. That other influences, which cannot be satisfactorily explained, may play a role is suggested when absorption is obtained with a group O blood which should show no absorption, and also when an A material behaves in its absorption like a B and vice versa. It is hoped that more subtle methods may check such errors. Especially, the use of group-specific antiserums as applied systematically by Boyd and Boyd in their experiments should prove helpful. With the material reported on in the present paper, this method, however, has not been employed. But the fact that it was possible to reduce the initial error on repeated examination in the majority of the cases, even without the application of antiserums, seems to demonstrate sufficiently the fundamental reliability of the absorption procedure. In view of the aforementioned reasons the erroneous results seem to carry much less weight and final significance than the positive findings.

Regarding the complexity of the biologic reactions which are involved in the tests, it would indeed be surprising if only positive results were obtained. It must be remembered that the absorption test is not a direct serologic reaction such as occurs, for example, with the Hektoen-Uhlenhut precipitin reaction or with the typing of fresh sam-

ples of blood. In the precipitin reaction and in the ordinary typing reaction definite serologic properties are readily visualized. With the absorption test the presence of agglutinogens can be demonstrated only indirectly, i. e., by their ability to absorb agglutinins and to reduce the titer of a serum of known strength. Furthermore, the objections made by Hirszfeld and Lewinsky must be taken into account, namely, that there are influences which act on the material itself and also on the substratum which holds the stain. These factors are obviously impossible to evaluate at all times.

In view of the rather large percentage of tests which gave erroneous results in the first typing, definite conclusions cannot be drawn from any single test even though the controls are working satisfactorily. From the practical standpoint, in those cases in which only minute amounts of blood or of seminal material are available for examination, the investigator should be cautious in his interpretation of the results and should make note of the fact that other corroborative tests could not be carried out for the purposes of checking the first result. Concerning the practical application of the absorption tests, the following may be added: Just as in cases of bastardy only certain combinations are of value for the decision of the court, similarly here, in the application of the absorption test, the expert in many instances can give a definite answer, whereas in a smaller number of cases the results cannot be introduced as evidence. Hirszfeld's co-worker Lewinsky [10] outlined the situation in a reserved manner. Although his position seems to be too conservative in view of the experiences reported in the last years from quite different sources, it seems worth while to quote his summary (translated from the German), which gives a precise survey of all possible objections to, and fallacies inherent in, the absorption test.

1. The group qualities are not individual characteristics. Therefore we cannot state that a certain blood stain definitely came from a certain victim but only that it might have come from such a source.

2. The examination is obviously without value if the suspect claims that the stain in question comes from himself and if he is of the same group as the victim.

3. In view of the progressive deterioration of agglutinins it is for the time being not always possible to recognize positively group O.

4. The factors which destroy the group substances are not understood sufficiently. The absence of the group substance cannot be interpreted as proof that a certain stain has not come from an individual either A or B. And for the same reason the presence of A or B substance may not exclude with absolute certainty the possibility of group AB. At the present time all that can be stated is that factor A or B has been found but not that the bloodstain belongs to group A or B.

5. Group specific differentiation is not limited to blood. Group substances are found in secretions and excretions of the body. They are present in saliva, sweat and urine, often in greater concentration than in the blood. If one considers the ease and frequency with which clothing, linen and similar articles are polluted

with the aforementioned substances, it can readily be seen how the determination of the group of the blood stains on such articles may be frustrated.

6. One must also consider the group-specific differentiation of material of animal origin and in this connection the similarity of group substances of animals to those of human beings.

7. Finally, the effects of sunlight, chemicals, dyes, etc., on agglutinogens are insufficiently understood. The uncertain effects of these factors do not permit at the present time any extensive application of the determination of bloodstains in the field of criminology.

One further obstacle should be mentioned: In the United States it is difficult in most jurisdictions to obtain blood from a suspect for testing without his consent. The opportunity may present itself to determine the group of his saliva, unwittingly deposited on the tip of a cigaret. In this connection the existence of nonsecretors of group-specific substances in saliva must be considered (Schiff [7]). For this reason it is doubtful whether a result obtained by such a method would be admitted as evidence in court. Notwithstanding these negative conclusions, the positive point should be stressed, namely, that the absorption test should be applied whenever the opportunity arises, even though the results may subsequently be excluded as evidence. Experience has shown that a positive finding—e. g., victim's blood of group B, defendant's blood of group A, and blood on the defendant's weapon of group B—is a considerable help to the police and the prosecuting attorney in corroborating their suspicion concerning the origin of the stain. The information obtained through the absorption test occasionally has been sufficient to obtain a confession from the alleged assailant. In certain cases of murder which have been described by Lattes [17] and Popoff,[18] the testimony of the defendant concerning the origin of a blood stain was found to be incompatible with the results of the absorption test, and in several instances the defendant, confronted with such evidence, readily confessed and related his participation in the crime. Such situations and also those which have arisen in cases of disputed paternity in which false accusations of paternity were withdrawn after the plaintiff learned that blood tests were to be applied would bear out the practical usefulness of the absorption test, even though use of the results as evidence in a court of law may not be warranted for the time being.

## SUMMARY

The absorption test was carried out on 109 dried blood specimens and 93 stains obtained from seminal material, which came from subjects whose blood groups were originally known. In 94.5 per cent of the

17. Lattes, L.: Deutsche Ztschr. f. d. ges. gerichtl. Med. **9**:402, 1927.

18. Popoff, N. W.: Deutsche Ztschr. f. d. ges. gerichtl. Med. **12**:415, 1926.

cases it was possible to determine correctly the group of the blood stains, and in 93.6 per cent of the cases this was possible with the seminal stains.

Of the 109 initial tests on blood, 14 (13 per cent), and of the 93 initial tests on seminal stains, 11 (12 per cent), yielded incorrect results. By repetition these mistakes could be brought down to 5.5 per cent.

The method employed in carrying out the tests, the probable reasons for the errors, and the possibilities for elimination of the errors in applying antiserums are discussed.

The value of the absorption test applied to blood and seminal stains for forensic purposes is explained.

# CAPILLARY RUPTURE WITH INTIMAL HEMORRHAGE AS A CAUSATIVE FACTOR IN CORONARY THROMBOSIS

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It is generally agreed now that arteriosclerosis, usually of a marked degree, is the underlying cause of bland thrombosis of the coronary arteries. The exact mechanism of the precipitation of a thrombus in these arteries is not known, although several hypotheses have been advanced. The stagnation or eddying of blood which results from stenosis of the lumen by arteriosclerotic plaques has been thought sufficient in itself, particularly when congestive heart failure is present, to cause the formation of thrombi. But if this were true one would expect coronary thrombosis to be a commoner lesion than it is. Besides the stenosing plaques, then, other lesions have been sought which might damage the lining endothelium sufficiently to precipitate thrombosis. Boyd [1] noted an acute inflammatory lesion within the intima in two cases and suggested this as a possible precipitating factor. Leary [2] observed defects in the intima at the site of thrombosis in some instances (in older persons), and because the adjoining thrombus contained atheromatous material he concluded that rupture of an atheromatous "abscess" into the lumen was the immediate cause of thrombosis in these cases. Both of these authors and others have described hemorrhage within the intima near the site of thrombosis, but they considered it the result either of rupture of the vasa vasorum due to inflammation (Boyd), or of back flow of blood from the lumen into the intima through a defect produced by the rupture of an atheromatous "abscess" (Leary).

In a previous paper [3] I suggested that intimal hemorrhage is due, rather, to rupture of capillaries which are derived from the coronary lumen; that capillary rupture results both from high intracapillary pressure and from softening of the supporting stroma by atheromatous degeneration, and, finally, because intimal hemorrhage was found at the site of occlusion in nine consecutive cases of recent coronary thrombosis,

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1. Boyd, A. N.: Am. J. Path. **4**:159, 1928.
2. Leary, T.: Arch. Path. **17**:453, 1934.
3. Paterson, J. C.: Arch. Path. **22**:313, 1936.

that it might be the precipitating factor in the formation of the coronary thrombus. I present now further and more definite evidence in support of this hypothesis.

## MATERIAL AND METHOD

The material was obtained in routine autopsies on adults over a period of approximately eighteen months. When either a discrete intimal hemorrhage or an occluding thrombus was found in a coronary artery, a varying number of blocks were cut. In the case of the discrete hemorrhage, sections were cut through the approximate center of the lesion and at various levels in each direction. In four instances, serial section at from 7 to 10 microns was done through the entire hemorrhagic area, every section being mounted and stained. When an occluding thrombus was encountered, as a rule blocks were taken through several parts of the thrombosed area, and a varying number of levels cut from each. The number of sections examined depended principally on the ease with which intimal hemorrhage was found in definite relation to the site of thrombosis. In six consecutive cases, however, the entire thrombosed portion of the artery was embedded and sectioned serially at 7 microns from one end to the other. When the thrombus was short, every section was stained and examined, but when it was long, sections were mounted at intervals varying from 175 microns upward. The great majority of the sections were stained with hematoxylin and eosin, while occasionally Perl's stain, Weigert's stain for fibrin, phosphotungstic acid, Mallory's connective tissue stain, a modified Weigert stain for elastic tissue and Glynn's bacterial stain[3a] were used. A longitudinal diagrammatic reconstruction of the thrombosed portion of the artery was then made to show the relation of the thrombus to various lesions in the intima (fig. 4).

## OBSERVATIONS

The hemorrhage into the intima of the arteriosclerotic coronary artery has a characteristic structure whether or not the adjacent lumen is occluded by a thrombus. That without associated thrombosis, however, is more readily studied, as it does not show the additional secondary changes which result from obstruction of the normal process of imbibition. The discrete intimal hemorrhage, without thrombosis of the immediately adjacent coronary lumen, has been found in thirty-one persons to date. It has been observed in two types of cases—in those in which there was no thrombosis of any part of the coronary system (fifteen cases) and in those in which occluding thrombi were found in the same heart but in parts of the coronary arteries distant from the discrete hemorrhage (sixteen cases). As discrete hemorrhages were sought in all routine autopsies during the eighteen months' period (approximately seven hundred autopsies), their relatively high incidence with coronary thrombosis is evident. Discrete intimal hemorrhages, with or without associated thrombosis, were frequently multiple; in one case single lesions were found in each of the three main coronary branches. The tendency toward multiplicity was more marked in persons with associated thrombosis.

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3a. Glynn, J. H.: Arch. Path. **20**:896, 1935.

In my previous paper, in the section dealing with the structure of discrete intimal hemorrhages, a detail of some importance was omitted: While it was stated that the endothelium overlying the lesion was intact both as viewed in the gross specimen and as observed microscopically, no evidence was submitted to prove that this was actually the case. At that time, however, one hemorrhage had been sectioned serially in its entire extent and no break in the endothelium or in the superficial intimal layers had been found. Recently, this procedure has been repeated on three occasions; in two cases a similar intact endothelium and connective tissue layer was found between the extravasated blood and the lumen of the artery, while in the other there was a small defect in the superficial tissues. A detailed account of the observations in one of these cases follows:

CASE 1.—A 73 year old man died two days after resection of the rectum for adenocarcinoma. Autopsy revealed extensive secondary growths in the liver and lungs and acute generalized peritonitis. The coronary arteries were slightly sclerotic, but no stenosing lesions were found. The right coronary artery, at a point 3.5 cm. from its origin, showed a small oval area of grayish brown discoloration of the intima, projecting slightly into the lumen of the vessel. The openings of two small branch arteries lay at one end of the hemorrhagic area. The lesion was removed in one piece and sectioned serially at 7 microns from one side to the other, the sections being cut longitudinally. All of the sections were stained and examined microscopically.

No break in the endothelium or in the superficial intimal layers was found in any of the sections. The lumen was free from evidence of deposition of a thrombus. The hemorrhage had occurred into the midintimal region (fig. 1 *A*), and here numerous foam cells were still visible. At each side of the hemorrhage and in the intimal layers superficial thereto were many capillary channels. These anastomosed, one with the other, and arose from the lumen by five distinct openings. Four of the points of origin were in the vicinity of the mouths of the two branch arteries (fig. 1 *B*), but one was from the lumen proper at the apex of the atherosclerotic swelling. The capillaries were most dilated as they entered the atheromatous focus, and at one point a dilated capillary actually penetrated the area of hemorrhage (fig. 1 *C*). A few capillaries which lay on the outer aspect of the hemorrhage appeared to anastomose with the vasa vasorum, but this evidence of capillary drainage from a vascularized plaque was not definite.

The intimal hemorrhage in this case could not have resulted from backflow or diffusion of blood from the lumen, as the intervening structures were intact and dense. The intimate association of capillaries with the extravasated blood suggests strongly that rupture of these channels was the cause of the hemorrhage. The capillaries arose from the main coronary lumen, and they were most dilated where they entered the atheromatous focus into which the hemorrhage had occurred. The other discrete hemorrhages in the series, whether studied by serial or by random section, had a similar structure. An area of

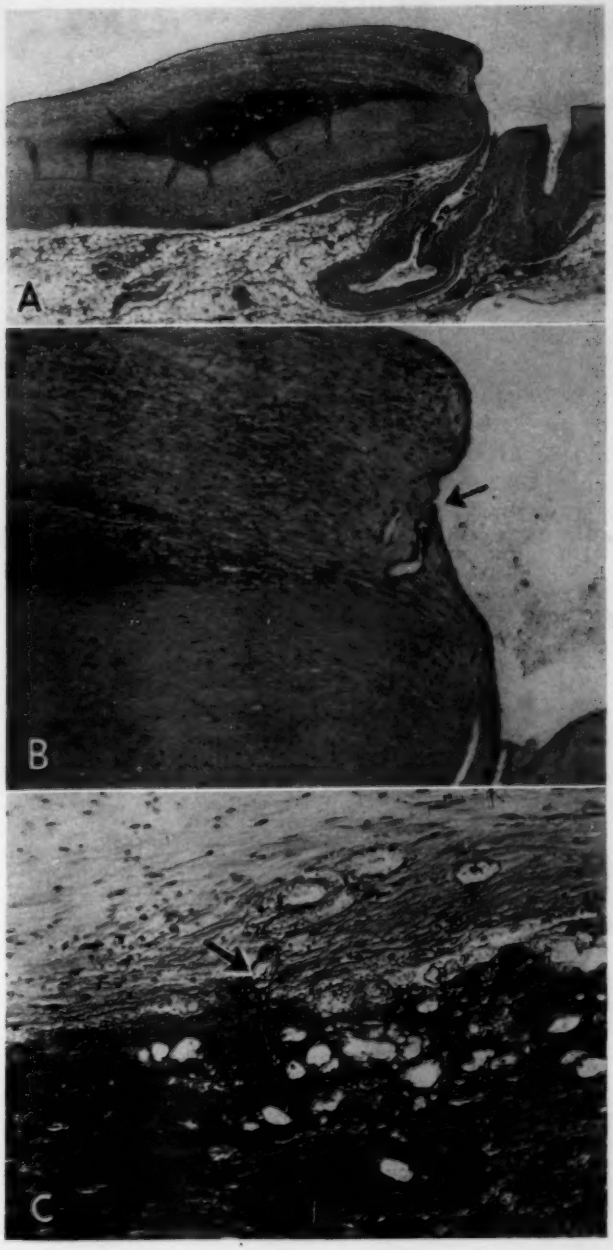


Fig. 1.—Photomicrographs of the discrete intimal hemorrhage described in detail in the text. *A* shows the position and extent of the hemorrhage; × 10. Note the thickness and density of the tissues between it and the lumen. *B* shows one of the capillaries arising from the lumen; × 60. *C* shows one of the intimal capillaries entering the area of hemorrhage; × 80. All the sections were stained with hematoxylin and eosin.

atheromatous degeneration was the constant site of the hemorrhage. Intimal capillaries, lying in close proximity to both the hemorrhage and the lumen of the artery, were usually seen, but they were most numerous when the hemorrhage was slight. In one case (fig. 2 *A*) the capillary ramifications through the inner two thirds of an atheromatous plaque were so extensive that they must be regarded as a true blood supply to the part. Indeed, it is difficult to conceive how the "necrosis" observed in the outer zone of this plaque could have been due to poor nutrition. In this case, one of the larger and more superficial intimal capillaries contained thrombus material (fig. 2 *B*). Similar capillary thrombi have been observed in proximity to intimal hemorrhages in other cases, including some in which there was thrombosis of the adjacent coronary lumen. Capillary thrombosis, of course, may be a primary lesion, but it is more reasonable to suppose that it results from an injury to the endothelial lining such as would be caused by rupture. These capillary thrombi are important in that should one propagate in a retrograde manner it might form the nucleus of an occluding clot in the main coronary lumen.

In the series of thirty-one discrete hemorrhages there were other lesions which, like intimal hemorrhage and capillary thrombosis, appeared to be secondary to capillary rupture. Erosion of the intima at the site of hemorrhage was observed in a number of cases, including one in which the hemorrhage was studied by serial section, but because erosion was absent in the majority it cannot be regarded as the primary lesion. Such defects in the intima develop apparently from nutritive necrosis, which is the direct result of interference with the capillary circulation, first from capillary rupture and later perhaps from retrograde capillary thrombosis. Inflammatory infiltration about the hemorrhagic focus was also an inconstant finding. It tended to be more intense when organization of the hemorrhage had begun and when there were necrotic changes in the adjacent tissues. Again, in two of the series, the hemorrhage into the intima was so massive that it appeared to compress further the already narrowed coronary lumen. The caliber of the lumen prior to capillary rupture could not be estimated, so the stenosing effect of intimal hemorrhage may have been more apparent than real. As a possible complication of capillary rupture, however, such an effect should not be completely ignored. Finally, it appears that coronary thrombosis results directly from intimal hemorrhage or from one of the other secondary lesions just described.

Intimal hemorrhages, similar microscopically to the discrete lesions, have been a common finding at sites of occlusion in cases of recent coronary thrombosis. During the eighteen months' period, thirty-seven

recently thrombosed arteries were studied, and in thirty-two of these hemorrhage into the intima was found at the site of occlusion. In sixteen of the cases, in addition, there was a discrete hemorrhage in some other part of the coronary system, and in several instances there were multiple hemorrhages elsewhere in the system. In almost every case the hemorrhage at the site of occlusion had occurred into an atheromatous

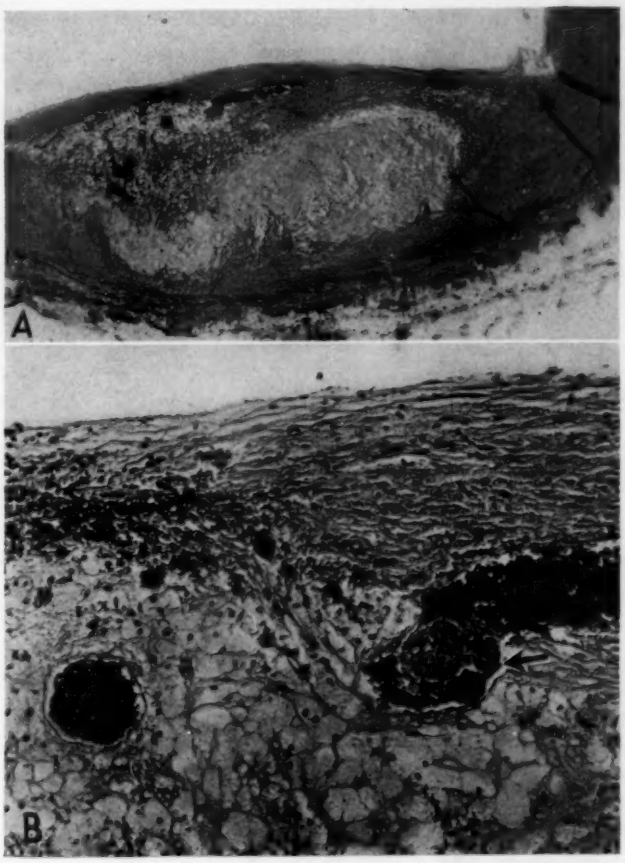


Fig. 2.—*A* is a section through a discrete intimal hemorrhage, showing the degree of vascularization present in some atherosclerotic plaques; × 10. *B* is the same section under higher magnification, showing the structure of the more superficial capillaries; × 80. The arrow points to a capillary thrombus. The section was stained with hematoxylin and eosin.

focus, and evidences of calcification in these regions were slight or entirely absent. Ramifying intimal capillaries, some of which were demonstrated arising from the coronary lumen at a point where it was

not occluded, lay in close proximity to the extravasated blood in the majority of the cases. The capillaries, on the whole, were not as numerous as those with the discrete hemorrhages, but many channels may have been involved in the necrosis which results from obstruction to imbibition (and to the origin of the capillary circulation) by occluding masses in the lumen. The incidence and degree of intimal hemorrhage and vascularization might have proved greater if serial section through the entire area of thrombosis had been done in each instance. This procedure, which has been recommended recently by Clark and his co-workers,[4] was followed in the latter part of the series in six consecutive cases, the observations on which follow.

CASE 2.—A 55 year old man, known to have arterial hypertension, died six days after the onset of an attack of severe precordial pain. Autopsy revealed marked coronary sclerosis, thrombosis of the proximal portion of the left anterior descending coronary artery, infarction of the septum and wall of the left ventricle, and aneurysm and rupture of the heart. Two discrete intimal hemorrhages were found in the right coronary artery at points 3.5 and 7 cm., respectively, from the origin of the vessel. The thrombosed portion of the left anterior descending coronary artery was sectioned horizontally at 7 microns through its entire length, every section being examined.

The oldest part of the thrombus lay just proximal to a point of marked stenosis of the lumen. Here, it was attached to one side of the wall of the vessel only, but at a higher level it completely filled the lumen and then extended up on all sides forming a cup. In the hollow thus formed and extending as high as the origin of the artery, the lumen was filled with more recent thrombus. Where the older part of the thrombus only partially occluded the lumen, i. e., at its distal extremity, it was attached to the intima on the side on which massive hemorrhage had occurred into an atheromatous focus (fig. 3*A*). At one point, the area of atheroma with its contained hemorrhage lay in close contact with the thrombus attachment. In the approximate center of the atheromatous area there were many widely dilated and engorged capillaries, and these connected with capillaries of smaller caliber in the inner and denser intimal layers. Some of the smaller channels arose from the lumen distal to the point of thrombosis, while other branches lay in close proximity to the lumen where it was occluded. Several of the latter contained thrombus material. Adjacent to the dilated capillaries in the substance of the plaque, massive hemorrhage was seen. This consisted in part of intact red cells and in part of organizing blood clot.

The only other interesting finding in the sections was a large intimal defect at a higher level. The margins of this defect were well demarcated and did not present the ragged appearance of a recently ruptured atheromatous "abscess." The upper extremity of the older thrombus was attached to the central part of the base of the defect (fig. 4*A*). It then swept over the lower brim of the defect and was continuous with the occluding thrombus at a lower level. The remainder of the defect contained recent thrombus and postmortem clot. From its structure and from its position in relation to the occluding thrombus, this defect was not regarded as the primary site of thrombus deposition.

4. Clark, E.; Graef, I., and Chasis, H.: Arch. Path. **22**:183, 1936.

CASE 3.—A 49 year old man gave a history of unusual and severe exertion more than a week before the onset of precordial pain. Death occurred suddenly four days after the commencement of the attack. Autopsy revealed coronary sclerosis, recent thrombosis of the right coronary artery, infarction of the wall of the left ventricle, and aneurysm and rupture of the heart. The thrombosed portion of the right coronary artery measured over 8 cm. in length. It was divided into twenty-two blocks, all of which were embedded and sections cut from each. It was anticipated that the older portion of the thrombus could thus be isolated and then studied by serial section. However, the entire length of the thrombus was of a similar structure, so satisfactory serial section could not be done. Sections were examined at intervals of about 250 microns.

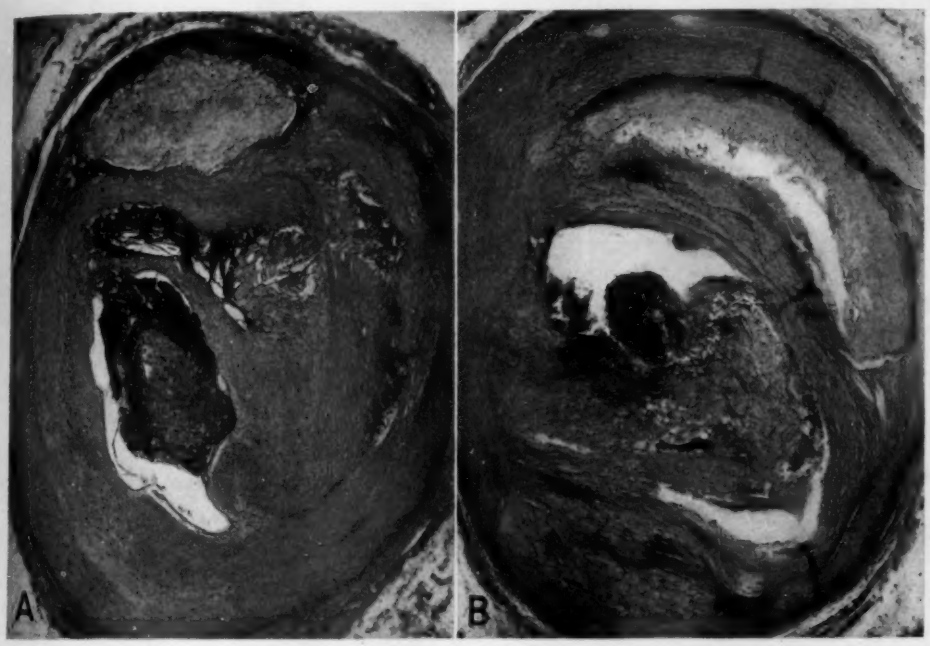


Fig. 3.—*A* is a section through the distal extremity of the thrombus in case 2; × 10. The thrombus occludes the lumen only partially, and it is attached in proximity to the hemorrhage in an atherosclerotic plaque. *B* is a section through the older portion of the thrombus in case 4; × 10. The close relationship between the partially occluding thrombus and the subendothelial hemorrhage is clearly shown. Fragments of younger thrombi are seen in other parts of the lumen. The sections were stained with hematoxylin and eosin.

The lumen was moderately stenosed at the distal extremity of the thrombus, and lesser grades of stenosis were seen at several higher points. The occluding thrombus, which consisted of two distinct segments, was attached for the most part to one side of the lumen, evidences of organization being most prominent on this side. Four points of superficial hemorrhage were found in the intima on the same side of the artery. At one point particularly, at the proximal end of

the more distal segment (fig. 4 *B*), the hemorrhage was in intimate contact with the thrombus. Here, the thrombus only partially occluded the lumen. Numerous engorged capillaries were visible in this hemorrhagic area. The hemorrhage had occurred into the inner aspect of an atheromatous focus. No atheromatous material was incorporated in the thrombus, nor was there microscopic evidence of intimal erosion. However, because of the unsatisfactory nature of the serial section, this negative finding was by no means proved.

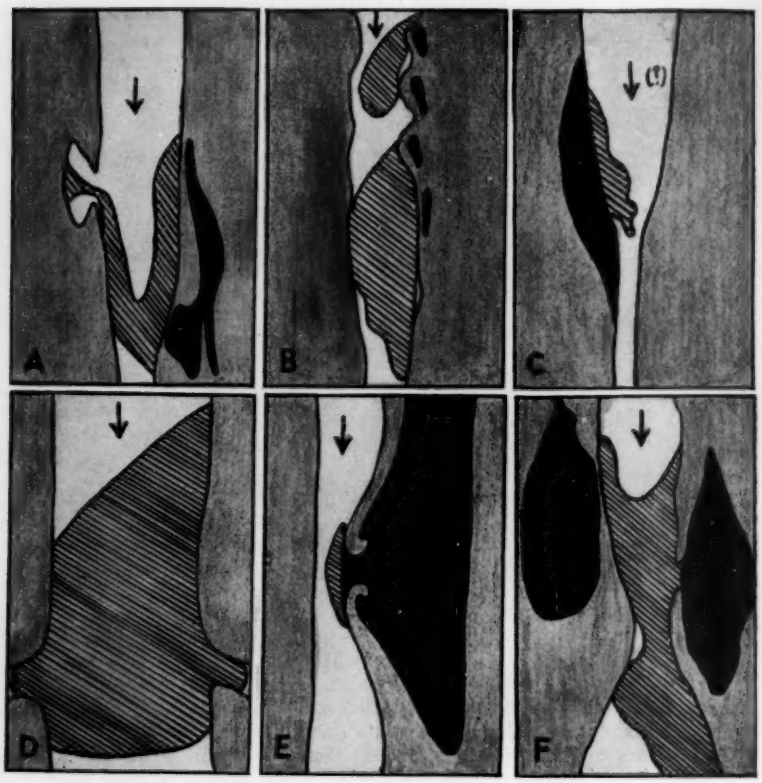


Fig. 4.—Longitudinal diagrammatic reconstruction of the thrombosed areas in cases 2 to 7 showing the relation of the coronary thrombi to various lesions in the intima. The diagonally shaded areas represent the oldest portions of the thrombi, while the solid black areas represent the position and extent of the intimal hemorrhages. The arrows show the direction of the blood flow. *A* represents case 2; *B*, case 3; *C*, case 4; *D*, case 5; *E*, case 6, and *F*, case 7.

Case 4.—A 54 year old man collapsed suddenly on the street and died before he could be hospitalized. No information as to his previous history was obtained. Autopsy revealed marked coronary sclerosis with recent thrombosis of the left anterior descending coronary artery. The left circumflex branch, at its immediate origin, showed a localized area of intimal hemorrhage without thrombosis of

the adjacent lumen. The thrombosed portion of the left anterior descending artery was 1.5 cm. in length. It was embedded in one block and sectioned serially at 7 microns from one end to the other. Every section was stained and examined.

The occluding thrombus lay on one side of a point of marked stenosis of the lumen, but the relation of the stenosing plaque to the direction of blood flow was not ascertained. The older part of the thrombus consisted of a small rounded radiating mass of condensed material, which was localized to one side of the lumen. The occluding process was completed by a more loosely arranged network of fibrin and platelets with enmeshed red cells. The older part of the thrombus was attached to the endothelium immediately opposite a point of marked hemorrhage into the inner aspect of an atheromatous focus (fig. 4 *B*). Serial section failed to reveal any break in the tissues lying between the intimal hemorrhage and the thrombus, nor was any atheromatous material incorporated in the latter. At the point of attachment of the older thrombus, a rounded structure resembling a thrombosed capillary was seen in the subendothelial tissue. Otherwise, there was little evidence of intimal vascularization.

CASE 5.—A 75 year old woman died three days after the onset of symptoms of acute intestinal obstruction. Autopsy revealed an incarcerated femoral hernia, slight coronary sclerosis, recent thrombosis of the right coronary artery and infarction of the myocardium. Serial section at 7 microns was done through the entire area of thrombosis. Where the thrombus was obviously recent, sections were mounted every 200 microns, but where it was older every section was examined.

There was no stenosis of the lumen, and only slight coronary sclerosis was found in the thrombosed area. The slight intimal thickening was of the fibrous variety, there being little evidence of atheromatous degeneration. No evidence of intimal vascularization or of intimal hemorrhage was seen in any of the sections. The only lesion found was a small subendothelial focus of necrotic tissue heavily infiltrated with fragmented leukocytes. No bacteria, calcium, hemosiderin or fibrin could be demonstrated in this lesion by special stains.

The precipitating factor of thrombosis in case 5 appeared to be a necrotic focus in the intima, the cause of which was obscure. The absence of intimal hemorrhage and vascularization excludes capillary rupture as the cause of the thrombosis; but because the arteriosclerosis was so slight and because there was no stenosis of the lumen, there is some doubt whether this lesion really belongs to the type of coronary thrombosis under discussion.

CASE 6.—A 67 year old woman, known to be hypertensive, had suffered from angina pectoris for fifteen years. She died suddenly three days after the onset of an attack of substernal pain typical of coronary thrombosis. Autopsy revealed marked sclerosis and stenosis of all of the coronary branches, recent thrombosis of the left circumflex artery, and infarction and rupture of the left ventricle. Serial section at intervals of 200 microns was done through the entire extent of the thrombus.

The most striking lesion microscopically was a massive hemorrhage into a large atheromatous focus which was localized to one side of the lumen. The lumen was markedly stenosed at the point of hemorrhage, but it was impossible to say whether this was a prior lesion or one due to the extravasation of blood. The apex of the stenosing plaque showed a small area of superficial erosion, which

was capped by a thin layer of organizing thrombus, in which some atheromatous débris was incorporated (fig. 4 *E*). Distal to this point, the lumen was filled with more recent thrombus. A number of small intimal capillaries were seen in the subendothelial tissues on each side of the eroded area.

CASE 7.—A 55 year old man, known to be hypertensive, suffering from carcinoma of the descending colon, had an attack of coronary thrombosis and died three weeks later. Autopsy revealed carcinoma of the sigmoid with secondary growths in the vertebrae, liver, adrenal glands and right lung, marked coronary sclerosis, old and recent thrombosis of the left anterior descending and left circumflex branches, respectively, and old and recent infarcts of the heart. The right coronary artery and the immediate origin of the left circumflex, in addition, showed three discrete intimal hemorrhages without thrombosis of the adjacent lumen. The recent thrombus in the left circumflex branch was studied by serial section, sections being examined at intervals of 175 microns.

The thrombus lay on each side of a point of marked stenosis. It completely occluded the lumen, and at its edges there was evidence of early organization. At several levels, masses of atypical epithelial cells arranged in pseudoglandular formations were incorporated in the thrombus. A large atheromatous focus encircled the lumen opposite the point of stenosis, and into it massive hemorrhage had occurred. The hemorrhage lay in close proximity to the lumen but also extended outward to occupy both the media and the inner layers of the adventitia. A small point of intimal erosion was noted just proximal to the stenosing plaque. Large numbers of capillaries were seen in various parts of the intima in proximity to the hemorrhage. Those in the outer and more degenerated parts were widely dilated and engorged with red cells, while those in the inner layers were smaller in caliber, and in several instances contained thrombus material.

Of the six cases studied by serial section, a marked degree of intimal hemorrhage was seen at the site of thrombosis in five. This figure conforms roughly to that obtained in the entire series, viz., thirty-two cases with intimal hemorrhage among thirty-seven, or 86 per cent. Although in some cases this lesion failed to appear it is obvious that for all practical purposes intimal hemorrhage is a characteristic finding in coronary thrombosis. The intimal hemorrhages associated with thrombosis were similar in structure to the discrete lesions previously described. Each of the five cases of thrombosis with hemorrhage showed intimal capillaries in close proximity to the extravasated blood. In three cases they were abundant, while in two they were scant. In one instance the capillaries were demonstrated arising from the main coronary lumen at a point distal to the occluding thrombus. They then invaded the intima, entered an atheromatous focus, and lay eventually in the area of hemorrhage. In the hemorrhagic region the capillaries were widely dilated. Evidence of capillary thrombosis was noted in three instances, and in one the thrombosed capillary lay at the point of attachment of the coronary thrombus. The intimal hemorrhage was confined to one side of the wall of the vessel in four cases, and in these a partially occluding mass of thrombus was attached on the same side at a point immediately overlying the hemorrhage. The lining endo-

thelium, and the superficial intimal layers lying between the hemorrhage and the thrombus were intact in three instances. The continuity of the intima was proved in two cases by serial section through the entire thrombosed area. The lapse of time between the moment of occlusion (as manifested by the onset of precordial pain) and the death of the patient had no relation to the degree of intimal hemorrhage as found at autopsy. For example, there was more hemorrhage in one patient who died suddenly on the street (case 4) than in another who survived for four days (case 3).

## COMMENT

From the foregoing observations, particularly those on serial sections, it appears that the two varieties of intimal hemorrhage—that with, and that without, thrombosis of the adjacent coronary lumen—are identical in structure and in origin. If this identity is granted, certain conclusions may be drawn. Intimal hemorrhage occurs at the site of thrombosis either from coincidence or as a result or as a cause of the occlusion. The factor of coincidence is improbable as intimal hemorrhage of some degree has been observed at the site of thrombosis in thirty-two of thirty-seven consecutive cases. Because discrete intimal hemorrhages have been found so often in coronary arteries with patent lumens, hemorrhage cannot be regarded as a secondary intimal change resulting from the presence of an occluding mass in the lumen. By a process of elimination, therefore, one can deduce that intimal hemorrhage, together with other lesions which appear to result from capillary rupture, is intimately concerned with the etiology of most coronary thrombi. The microscopic evidence confirms this deduction. One of the most striking observations in this series was the fact that when a thrombus only partially occluded the lumen (as in cases 2 and 4) it was attached on that wall of the vessel into which the hemorrhage had occurred.

Because intimal hemorrhage occurs so often with complete patency of the adjacent lumen, it is equally clear that some additional factor is necessary to initiate thrombosis. The only other common finding in the series was the presence of stenosis of the lumen at a point distal to the site of the attachment of the thrombus. Clark [4] called attention to this same peculiar location of stenosing arteriosclerotic plaques in relation to coronary thrombi, and such a finding appears to be characteristic. It is concluded, therefore, that if the proper conditions of stagnation and eddying of blood exist at a given point in the coronary system, capillary rupture with its sequelae occurring in the same region may precipitate thrombosis. The mechanism may vary in individual cases. 1. When the hemorrhage is superficial, there may be diffusion of blood and

thromboplastic substances from the intima into the lumen. 2. The rupture of the nutrient capillaries may be so extensive that necrosis of the intima results. The necrosis may actually involve the endothelium, or the pressure of blood within the lumen may rupture the thin shell of viable tissue, thus producing a defect with a raw surface. 3. When the hemorrhage occurs into the deeper intimal layers, the capillaries adjacent to the point of rupture may thrombose; retrograde thrombosis may then take place, and, when the process reaches the origin of the capillary, the thrombus may form the nucleus of an occluding mass in the lumen of the coronary artery.

The fact that in one of the cases studied serially there was absolutely no trace of hemorrhage or vascularization in the thrombosed region must mean that other lesions, such as necrotic foci of obscure origin, are occasionally at fault. Necrotic foci in the absence of hemorrhage have been noted in only two instances in the entire series (and absence of hemorrhage proved in only one), so coronary thrombosis precipitated by this lesion must be rare. Erosions of the intima and infiltration with inflammatory cells (in association with intimal hemorrhage) are often present in individual cases, but because they are absent in others they must be regarded as secondary changes.

The mechanism of capillary rupture has been dealt with in detail elsewhere.[3] Two principal factors appear to be involved, (1) softening, by atheroma, of the supporting stroma and (2) high intracapillary pressure. Intimal hemorrhage occurs commonly into those parts which are affected by atheromatous degeneration. It is assumed that softening, which is a physical character of atheroma, allows the pressure of blood within the capillary to dilate its walls to the extent that rupture eventually occurs. This assumption is borne out by the fact that intimal capillaries are usually of small caliber when seen in the denser intimal layers, while they are frequently dilated in areas of atheroma. Again, the age of incidence of coronary thrombosis corresponds roughly with that in which atheroma usually develops, i. e., late middle age. Younger patients, with characteristically dense and fibrous arteriosclerotic lesions, and elderly ones, with heavily calcified plaques, are not so prone either to intimal hemorrhage or to coronary thrombosis. The factor of high intracapillary pressure appears to be equally important. Intimal capillaries, because they arise directly from the main coronary lumen, are exposed constantly to a relatively high pressure of blood. When the arterial blood pressure is persistently high, as it is in approximately 50 per cent of the cases of coronary thrombosis prior to occlusion, or when it is elevated temporarily during excitement or exertion, the danger of capillary dilatation and rupture, it is reasonable to suppose, will be increased. Some patients with coronary thrombosis

give a history of unusual physical exertion or excitement many hours or even days before the onset of precordial pain. It is during that time of stress that capillary rupture probably occurred, the ensuing latent period being occupied by secondary changes in the intima which led eventually to the deposition of a thrombus. The onset of precordial pain, then, merely marks the time of coronary occlusion which is the final phase of a process initiated by capillary rupture some time previously.

## SUMMARY

Hemorrhages into the intima of sclerotic coronary arteries have been shown by study of serial sections to be intrinsic lesions and not the result of intimal erosions. They are due, apparently, to rupture of capillaries which are derived from the coronary lumen. Intimal hemorrhages have been observed both with and without thrombosis of the adjacent coronary lumen, and in either case they are identical in structure. Intimal hemorrhage has been a common finding in recently thrombosed coronary arteries, hemorrhage of some degree having been found in thirty-two of thirty-seven consecutive cases. The microscopic observations, particularly in those cases in which the thrombus only partially occluded the lumen, suggest strongly that intimal hemorrhage (or the other sequelae of capillary rupture) is intimately concerned with the etiology of most coronary thrombi. It is concluded that, if the proper conditions of stagnation and eddying of blood exist at a given point in the coronary system, capillary rupture with its sequelae occurring in the same region may precipitate thrombosis.

Capillary rupture may initiate thrombosis of a coronary artery by diffusion of the blood from an intimal hemorrhage into the lumen, by necrosis or erosion of the intima from damage to its capillary circulation or by retrograde capillary thrombosis. Any one or all of these factors may operate in an individual case.

# EFFECT OF CERTAIN ARSENATES ON THE LIVER

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Though arsenic has long been known to cause extensive damage in the liver, there have been but few experiments recorded in which cirrhosis was produced with it.

The earliest reported experiment was that of Ziegler and Obolonsky,[1] in 1888. They administered small doses of arsenic subcutaneously and by mouth to dogs and rabbits. In the rabbits fatty change of the liver cells in the central part of the lobule was observed after a few days; at the end of 9 days shrinking and hyaline degeneration of individual liver cells, with fragmentation of the nuclei, were present. After 14 days, in addition to fine fat droplets, there were necroses, the necrotic liver cells having a spongy appearance. In the livers of the rabbits that lived 16 days, small groups of swollen liver cells without nuclei, an increase of connective tissue and proliferation of bile ducts were found. One of the rabbits survived for 20 days. In addition to the alterations described, many multinucleated cells were present in the sinusoids. Another rabbit remained alive for 25 days; degeneration of single hepatic cells and numerous giant cells were seen. The only hepatic lesion produced in dogs following the administration of arsenic in doses of from 0.01 to 0.1 Gm. over a period of 90 days was vacuolar degeneration of liver cells and fatty infiltration. The fat was not abundant as in the livers of the rabbits.

Podwyssotzky,[2] in the same year, reported somewhat similar results in guinea pigs after subcutaneous injection of sodium arsenite in amounts of from 0.005 to 0.01 Gm. Some of the animals lived but a short time—from 3 to 6 days. Necrotic areas were found in the livers, the connective tissue was increased, and bile ducts were proliferating. Mitoses were present in fibroblasts and the epithelium of bile ducts. Later the masses of necrotic liver cells were sharply delimited, as though sequestrated, and were invaded by newly formed bile ducts and connective tissue. In the animals whose period of survival was from 15 to 25 days, the necrotic liver cells had disappeared for the most part and were replaced by recently formed liver cells and scar tissue.

Wolkow[3] administered a solution of potassium arsenite (Fowler's solution) by subcutaneous injection to eighteen rabbits. Doses of from 0.003 to 0.005 Gm. of

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1. Ziegler, E., and Obolonsky, N.: Beitr. z. path. Anat. u. Physiol. **2**:291, 1888.
2. Podwyssotzky, W. W., Jr.: St. Petersburg. med. Wchnschr. **5**:211, 1888.
3. Wolkow, M.: Virchows Arch. f. path. Anat. **127**:477, 1892.

arsenious acid were well tolerated; following large doses of from 0.01 to 0.02 Gm. the animals lived only a few days. Nine of the animals were put to death at varying intervals, the longest period being 36 days. The other nine rabbits were allowed to succumb from the effects of the arsenic. In this group the longest period of survival after the beginning of the experiment was 60 days.

The most constant finding was fatty degeneration of the liver cells and Kupffer cells, usually in all parts of the lobule. Areas of necrosis of liver cells were found as early as the second day in five of the animals given the larger doses. The necroses were situated most often at the periphery of the lobule; the necrotic cells were frequently swollen, the cytoplasm was clear and transparent, and their nuclei stained faintly and were scarcely visible. Leukocytes had collected at the periphery of each area of necrosis and had also penetrated between the necrotic cells. These foci of necrosis were sharply defined from the surrounding cells. The stroma remained in the necrotic areas; fine fat droplets were not conspicuous in the necrotic cells. Mitoses were observed in a few of the liver cells, and in three of the animals multinucleated liver cells were found. Inflammatory reactions in the bile ducts were moderately frequent. The connective tissue of the portal areas was increased in three animals, and bile ducts were proliferating.

Ogata [4] reported changes in the liver following administration of icterogen (a powerful hepatotoxic organic arsenical preparation). Necroses were found peripherally in the lobules of the livers of guinea pigs, and macrophages were numerous in some. The animals lived but a few days after the injections. Similar necroses occurred in the livers of rats, and in those that survived as long as 28 days there was severe cirrhosis. Identical lesions were produced in the livers of mice. No lesions were discovered in the livers of rabbits.

The most recent report is that of Stoeber,[5] who injected intravenously sodium arsenite in neutral solution. Rabbits were used in the experiment; some of the animals lived 7½ months. In the animals in which the poisoning was subacute, there were observed swelling, hemosiderosis of the Kupffer cells and also phagocytosis of erythrocytes by these cells. More striking were enlarged pigment-containing phagocytes at the periphery of the lobule and in the portal areas. The pigment held within the phagocytes was in part iron containing. The portal areas were widened by fibroblasts and fibrocytes, and bile ducts were proliferating. Small areas of necrosis involving a few liver cells were found scattered throughout the lobule but were more frequent close to the efferent vein and portal area. The Kupffer cells were well preserved in these areas. After from 3 to 6 weeks the liver cell layer bordering the portal area was irregular. The epithelial cells of the precapillary bile ducts contained fine droplets of fat, and these ducts were surrounded by sparse numbers of leukocytes. In the long-continued experiments, lumens developed in the proliferated bile ducts, and single liver cells were separated from the lobule. When the poisoning was chronic, the hepatic lobules were surrounded by broad bands of fibrocytes and histiocytes, in which were proliferating bile ducts and large groups of liver cells. Fat infiltration of the liver was found in both the acutely and the chronically poisoned animals. Of the eighteen rabbits used in this experiment, five did not show cirrhosis; in ten there was beginning cirrhosis; of the other three animals, one had cirrhosis, one necroses, and one necroses and beginning cirrhosis. The arsenic content of the livers varied from 0.17 to 3.18 mg. per hundred grams.

4. Ogata, T.: Beitr. z. path. Anat. u. z. allg. Path. **55**:236, 1913.

5. Stoeber, E.: Beitr. z. path. Anat. u. z. allg. Path. **97**:367, 1936.

A second group of rabbits was given intravenous injections of the arsenical compound and from 20 to 30 per cent alcohol by stomach tube in amounts of from 10 to 50 cc. Some of the animals died within a short time. The livers of the animals that lived for a longer period were small, and the consistence was somewhat increased. Numerous areas of necrosis were present; the necrotic liver cells had a netlike transparency as though only the cell membranes remained. Whether the necroses healed without traces could not be determined. The Kupffer cells in the necrotic foci were unaltered. In an occasional animal there were more widespread nuclear changes, similar to those found in acute yellow atrophy; the liver cells were larger than normal, the nuclei were pyknotic or had disappeared, and in some cells there were double nuclei. The increase of connective tissue in the portal areas began in a shorter time and was more marked than when the arsenical compound was given without alcohol. At the end of from 2 to 3 months the portal areas were markedly widened and bile ducts were proliferating. Two of the rabbits lived between 6 and 7 months. The changes in the livers are described as being similar to those of the early stages of Laënnec's cirrhosis. Large groups of liver cells and single liver cells were cut off from the lobule. Thirteen rabbits were used in the experiment. No lesions were found in three; five had beginning cirrhosis; in one there were necroses in the liver, and the other four had cirrhosis. Two of the four with cirrhosis also had necroses in the liver. From 0.74 to 2.43 mg. of arsenic per hundred grams of liver was found, while in the bile the arsenic content was from 0.004 to 0.06 mg. per hundred cubic centimeters. It was not believed that there was any relationship between the lesions produced and hemochromatosis. The alcohol was thought to have affected or increased the effect of the arsenic.

## MATERIALS AND METHODS

During a study of the effect of various diets on the liver of the rabbit a series of ten animals was fed on cabbage only. Pigment was increased in the liver cells of most of the animals, and in many of them large phagocytes containing pigment were present in the sinusoids. The experiment was repeated three times, but similar changes were not produced. Thinking that perhaps the cabbage fed to the first series of rabbits might have been treated with one of the insecticides, we decided to test the effect of arsenates on the liver. Cupric arsenate and lead arsenate, the two preparations commonly used as insecticides, were selected. The copper arsenate was chemically pure. As lead arsenate, one of the preparations sold for the purpose of spraying or dusting plants was used; it was 98 per cent lead arsenate. Sodium arsenate (chemically pure) was used to control any changes that might result from the copper or the lead in the other two compounds.

The dose of arsenate to be administered was small and tedious to weigh; it was found simpler to mix thoroughly the various arsenates with starch in such proportions that a given quantity of each mixture contained approximately an equal amount of arsenic.

Rats, ferrets and rabbits were used for the experiment. With the exception of those on the control diet of white bread and potatoes, the rabbits were of the chinchilla variety and weighed from 1,250 to 1,500 Gm. at the beginning of the experiment. Each rabbit was kept in a separate cage. From each rabbit except one a piece of liver was removed before the experiment was begun, and the arsenate was not given until from 10 to 14 days had elapsed after the operation. The incidence of coccidiosis was low; any animal with a moderately heavy infection was discarded.

The biopsy specimens were fixed in solution of formaldehyde U. S. P. (1:10). The material obtained at necropsy was placed in solution of formaldehyde U. S. P. (1:10) and in Zenker's solution to which neither solution of formaldehyde nor acetic acid had been added. Sections were stained with hematoxylin and eosin and with the stain for the differentiation of hemosiderin and hemofuchsin (Mallory, Parker and Nye[6]). Many sections were also stained with azan-carmine (McGregor[7]) and by the silver impregnation method described by Foot and Mènard.[8] In addition, numerous frozen sections of the liver were stained with sudan III.

Various diets were used as will be indicated. Any effect that might be attributed to the diet was adequately controlled by a group of rabbits fed the same diet and not receiving the arsenates.

An analysis of a sample from some cabbage to be used in one of the diets revealed 4.32 mg. of arsenic per kilogram. To prevent the animals from receiving a possible additional and unknown quantity of an arsenical insecticide with which the cabbage might have been treated, the leaves were separated and washed in running water for from 15 to 30 minutes. They were then placed in 1 per cent hydrochloric acid solution for 15 minutes and again washed in running water. This is the method recommended for the removal of arsenate from fruit. Numerous analyses of the livers and kidneys were made to determine the quantities of arsenic, lead and copper present. The amount of arsenic was determined by the Gutzeit method, the copper by the electrolytic method and the lead by the diphenylthiocarbazone method. To eliminate traces of arsenic and lead, all reagents used in the determinations were repurified, with the exception of the hydrogen peroxide, which was frequently tested for these two metals, but none was found.

### DIET OF HAY AND OATS

Forty-six rabbits were divided into three groups; in each of two groups were fifteen animals, and in the third, sixteen. Each rabbit of one group of fifteen was fed copper arsenate daily; the dose administered to ten of them was 5.6 mg. (1.4 mg. of arsenic). The other five (61, 62, 67, 68 and 70) received a like dose for the first 148 days. The daily amount was then increased to 9.3 mg. (2.33 mg. of arsenic) for the remainder of the experiment (table 1).

To each rabbit of the second group of fifteen, 6.18 mg. of sodium arsenate (1.5 mg. of arsenic) was given each day.

The other sixteen animals received lead arsenate, the dose each day being 7.2 mg. (1.4 mg. of arsenic) except that the dose of this arsenate for five of the rabbits (71, 74, 78, 79 and 80) was raised to 12 mg. (2.33 mg. of arsenic) after 132 days, and this daily amount was administered until they died.

6. Mallory, F. B.; Parker, F., Jr., and Nye, R. N.: J. M. Research **42**:461, 1921.

7. McGregor, L.: Am. J. Path. **5**:545, 1929.

8. Foot, N. C., and Mènard, M. C.: Arch. Path. **4**:211, 1927.

The appropriate arsenate was mixed with a small quantity of moistened oats. When these oats were eaten, a liberal quantity of oats and an unrestricted amount of hay were given. The animals readily ate the oats mixed with the arsenate.

TABLE 1.—*Observations on Rabbits Fed Arsenates with a Diet of Hay and Oats*

| Rabbit | Days | Cirrhosis of Liver | Content of Mineral Element in Liver, Mg. per 100 Gm. of Dry Weight | | | Content of Mineral Element in Kidney, Mg. per 100 Gm. of Dry Weight | | |
|---|---|---|---|---|---|---|---|---|
| | | | Arsenic | Lead | Copper | Arsenic | Lead | Copper |
| | | **Rabbits Given Copper Arsenate** | | | | | | |
| 101 | 16 | None | 0.254 | ..... | 1.223 | ..... | ..... | ..... |
| 105 | 48 | Severe | 0.923 | ..... | 2.460 | 18.640 | ..... | ..... |
| 104 | 67 | Severe | 2.346 | ..... | 3.216 | 0.940 | ..... | ..... |
| 103 | 72 | Early | 1.023 | ..... | 1.387 | 0.500 | ..... | 5.724 |
| 102 | 105 | Moderately advanced | 2.173 | ..... | 2.160 | 4.444 | ..... | 6.604 |
| 65 | 140 | Moderately advanced | 1.373 | ..... | 1.587 | ..... | ..... | ..... |
| 64 | 155 | None | 0.200 | ..... | 1.283 | ..... | ..... | ..... |
| 67 | 190 | Moderately advanced | 3.256 | ..... | 1.250 | 5.900 | ..... | 6.756 |
| 63 | 205 | Moderately advanced | 10.700 | ..... | 0.543 | 7.760 | ..... | ..... |
| 69 | 205 | Moderately advanced | 1,983 | ..... | 1.320 | 2.108 | ..... | 11.292 |
| 70 | 211 | Moderately advanced | 0.373 | ..... | 1.570 | 0.307 | ..... | 10.016 |
| 62 | 220 | Moderately advanced | 0.463 | ..... | 2.526 | 0.880 | ..... | 8.000 |
| 61 | 259 | Severe | 11.510 | ..... | 0.650 | 2.436 | ..... | 3.512 |
| 66 | 393 | Early | 0.259 | ..... | 1.146 | ..... | ..... | ..... |
| 68 | 759 | None | 0.067 | ..... | 8.900 | 1.600 | ..... | ..... |
| | | **Rabbits Given Lead Arsenate** | | | | | | |
| 2B | 76 | Moderately advanced | 3.280 | 0 | 3.100 | ..... | ..... | ..... |
| 114 | 95 | Severe | 3.216 | ..... | 1.930 | 1.668 | ..... | 2.232 |
| 112 | 100 | Severe | 0.926 | 0 | 0.783 | 4.444 | ..... | 7.000 |
| 111 | 111 | Moderately advanced | 5.210 | 0 | 2.936 | 0.596 | ..... | 6.088 |
| 113 | 113 | Severe | 2.776 | 0 | 3.890 | 0.932 | ..... | 6.932 |
| 72 | 125 | Moderately advanced | 0.167 | 0 | ..... | ..... | ..... | ..... |
| 79 | 167 | Severe | 20.420 | ..... | ..... | ..... | ..... | ..... |
| 74 | 180 | Early | 16.960 | 3.643 | 1.170 | 15.880 | ..... | ..... |
| 115 | 181 | Moderately advanced | 1.693 | ..... | 1.537 | 3.636 | ..... | 5.508 |
| 80 | 196 | Early | 1.160 | ..... | 3.600 | 0.444 | ..... | 4.712 |
| 71 | 205 | Moderately advanced | 7.956 | 0 | 0.723 | 3.180 | ..... | ..... |
| 78 | 209 | Early | 2.793 | Trace | 4.990 | 1.904 | ..... | 12.128 |
| 77 | 210 | Severe | 1.530 | 0 | 1.216 | 2.104 | ..... | 5.600 |
| 81 | 215 | Moderately advanced | 1.710 | 0 | 0.586 | 0.816 | ..... | 5.306 |
| 75 | 229 | Moderately advanced | 5.700 | 0 | 0.873 | 4.212 | ..... | 3.128 |
| 76 | 237 | Severe | 3.686 | 0 | 0.436 | 4.124 | ..... | 4.576 |
| | | **Rabbits Given Sodium Arsenate** | | | | | | |
| 121 | 3 | None | 4.883 | ..... | 0.663 | ..... | ..... | ..... |
| 124 | 52 | Early | 2.613 | ..... | 2.183 | 1.428 | ..... | 7.668 |
| 125 | 53 | Early | 3.247 | ..... | 2.370 | 3.836 | ..... | 11.560 |
| 123 | 59 | Early | 0.530 | ..... | 1.546 | 0.600 | ..... | 9.000 |
| 53 | 86 | Moderately advanced | 1.540 | Trace | 2.049 | ..... | ..... | ..... |
| 54 | 96 | Moderately advanced | 2.650 | 0 | 2.713 | ..... | ..... | ..... |
| 122 | 97 | Moderately advanced | 5.233 | ..... | 1.306 | 2.860 | ..... | 5.484 |
| 57 | 98 | Severe | 1.093 | ..... | 3.290 | ..... | ..... | ..... |
| 82 | 153 | Severe | 0.383 | ..... | 2.093 | ..... | ..... | ..... |
| 59 | 161 | Moderately advanced | ..... | ..... | 2.027 | ..... | ..... | ..... |
| 51 | 176 | Moderately advanced | 3.430 | ..... | 1.670 | ..... | ..... | ..... |
| 50 | 196 | Early | 19.570 | ..... | 2.190 | ..... | ..... | ..... |
| 56 | 226 | Early | 1.053 | ..... | 2.070 | 0.440 | ..... | 6.864 |
| 55 | 228 | Moderately advanced | 1.213 | ..... | 1.190 | 1.904 | ..... | 5.952 |
| 52 | 251 | Moderately advanced | 5.230 | ..... | 1.300 | 6.036 | ..... | 3.968 |

The lesions in the liver resulting from the ingestion of the various arsenate were so similar that a detailed description for each group of animals is unnecessary. As early as the third day after ingestion of the arsenate necroses involving liver cells were found. These necroses were sharply defined from the surrounding liver cells; the necrotic cells were

swollen and pale staining and somewhat rigid in appearance. In many instances the cytoplasm in the necrotic cells was transparent and did not stain, though the cell membrane was very distinct (fig. 1 *A*). The nuclei of many of these necrotic cells were pyknotic or fragmented; in others no nucleus could be discerned. There was no demonstrable fat in the necrotic cells.

The necroses were found in all parts of the lobule but were most often situated close to the portal area. Sometimes the necroses completely surrounded the portal area; in other instances they extended from one portal area to another, and somewhat less frequently from portal area to efferent vein. Less commonly they occupied a midzonal position or involved liver cells in the immediate vicinity of the efferent vein. Frequently two necroses situated against a portal area came into contact with each other, isolating a small group of intact liver cells from the remainder of the lobule. Occasionally either end of a mass of necrotic cells would touch a portal area and arch over a group of undamaged hepatic cells. In a few instances individual liver cells in all parts of the lobule were necrotic. At times intact bile ducts projected into the center of a mass of necrotic liver cells (fig. 1 *B*). Such ducts appeared to be unaltered.

About these areas of necrosis, there often accumulated large phagocytic cells; many of these were multinucleated. Similar phagocytes were frequently seen in the sinusoids and portal areas. The endothelial cells of the sinusoids were preserved in the areas of necrosis. Polymorphonuclear leukocytes were seldom found in the necrotic foci or portal areas, and when present about the necroses, were few.

After the necrotic liver cells were removed, there remained a moderately compact mass of delicate fibrillar material that appeared to be the collapsed stroma (fig. 1 *C*). Later, fibroblasts penetrated into the area (fig. 1 *D*). Since most of the necroses were immediately adjacent to the portal areas, this collapse of the stroma and new growth of connective tissue caused a widening of the portal area. Often these scars were continuous from one portal area to another, sharply accentuating the lobular pattern; in other instances they extended into the lobule for variable distances even to the afferent vein (fig. 2 *A* and *B*). Groups of intact liver cells were seen lying within the widened portal areas. Proliferation of bile ducts was often observed, and in many instances this new growth of ducts was marked. Phagocytes apparently remained in the scars for a considerable time, for they were found after all of the necrotic liver cells had disappeared. Mitoses were not seen in the liver cells; infrequently an enlarged liver cell contained two nuclei, but evidences of regeneration were slight. Pigment was always present in the

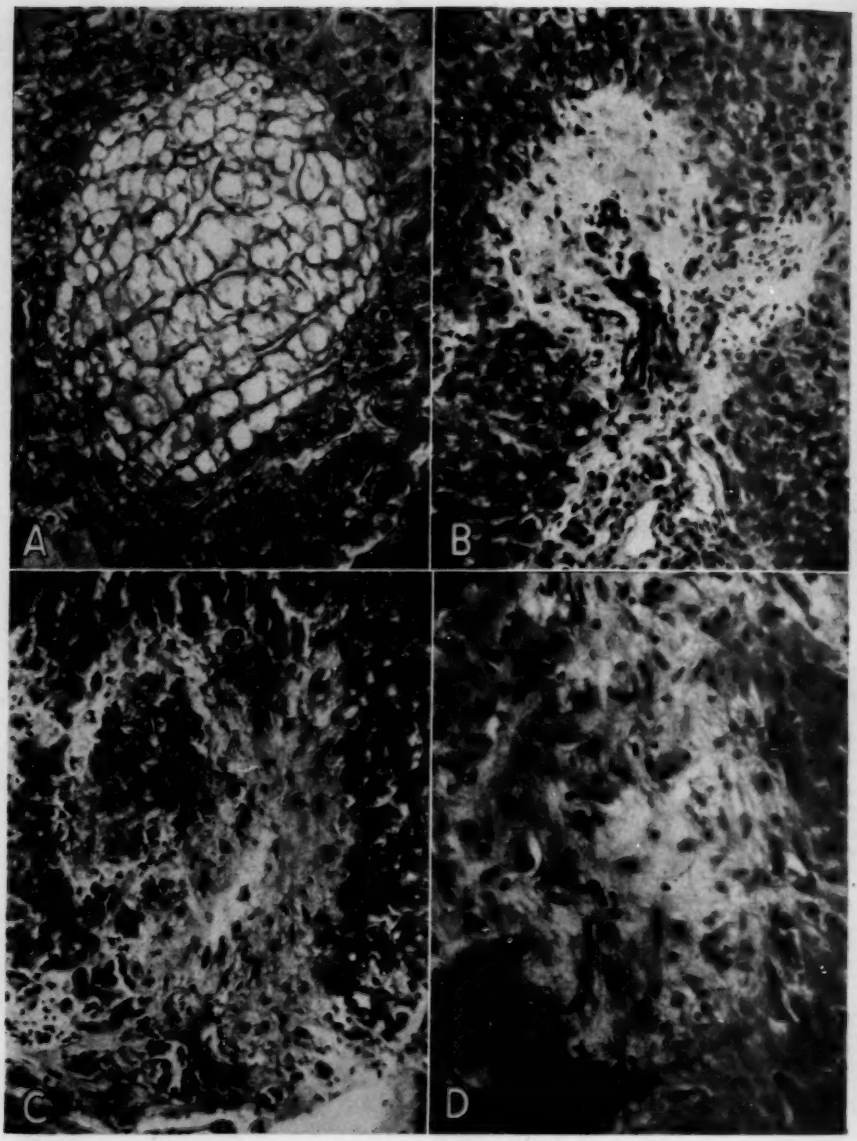


Fig. 1.—*A,* focal necrosis in the liver of rabbit 121, fed a diet of hay and oats and given sodium arsenate, 6.18 mg. Death occurred on the third day. Hematoxylin-eosin stain. *B,* bile duct projecting into an area of necrosis in the liver of rabbit 105, fed a diet of hay and oats and given copper arsenate, 5.6 mg. daily for 48 days. Hematoxylin-eosin stain. *C,* healing necrosis in the liver of rabbit 52, fed a diet of hay and oats and given sodium arsenate, 6.18 mg. daily for 251 days. Hematoxylin-eosin stain. *D,* healed necrosis in the liver of rabbit 112, fed a diet of hay and oats and given lead arsenate, 7.2 mg. daily for 100 days. Hematoxylin-eosin stain.

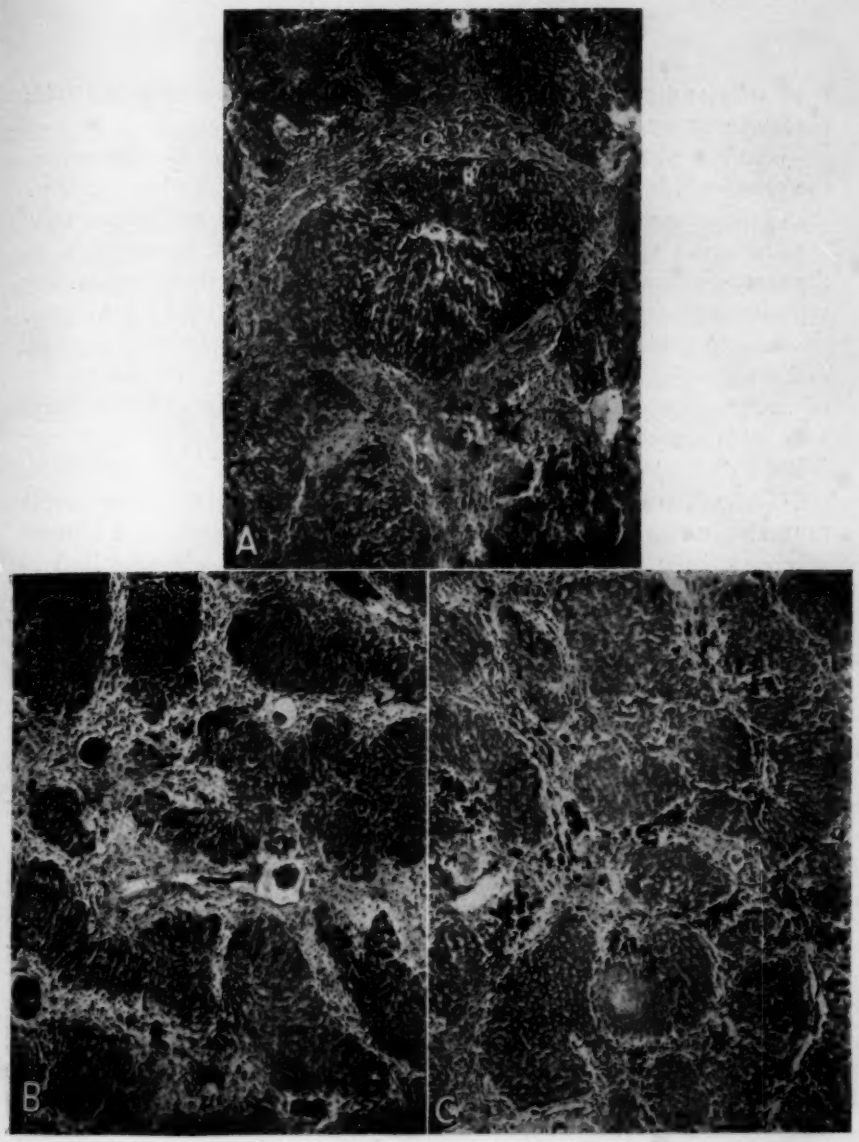


Fig. 2.—*A*, moderately advanced cirrhosis in rabbit 71. This animal was fed a diet of hay and oats and given lead arsenate, 7.2 mg. daily for 132 days; the dose of lead arsenate was then increased to 12 mg. Death occurred in 205 days. Connective tissue passes from one portal area to another, accentuating the lobular outlines. Hematoxylin-eosin stain. *B*, severe cirrhosis in rabbit 79. This animal was fed a diet of hay and oats and given lead arsenate, 7.2 mg. daily for 132 days; the dose of lead arsenate was then increased to 12 mg. The animal died in 167 days. The liver lobules are of irregular size and outline. Hematoxylin-eosin stain. *C*, severe cirrhosis in rabbit 127. The animal was fed a diet of hay and oats, carrots and cabbage and given sodium arsenate, 6.18 mg. daily for 23 days. The liver lobules are irregular in size and outline. There are many phagocytes filled with pigment in the portal areas. Fuchsin stain.

liver cells and phagocytes and frequently in the Kupffer cells. This pigment was granular, yellow and refractive.

Jaundice was present in nine of the livers, plugs of bile distending the canaliculi, but it was never sufficiently marked to be detected other than microscopically. Glycogen was markedly reduced in the liver cells.

The gross appearance of the liver was altered in twenty-eight of the forty-six animals. Many of the livers were smaller than normal. The capsular surface was slightly irregular in some; in others it was finely or coarsely pebbled. The resistance on sectioning was often increased, and irregularity of lobulation was frequently noted. The cut surface of many of these livers appeared moist and was gray and translucent over wide areas; usually in those animals that survived a longer time it had a distinctly brownish tint.

Cirrhosis was found in twelve of the fifteen rabbits given copper arsenate; in two it was early, in seven moderately advanced and in three severe. Of the three animals that did not have cirrhosis, one lived 16 days, another survived 155 days, and the third was put to death after 759 days. Necroses were present in the liver of the rabbit that lived 155 days; none was found in the other two livers.

Of the fifteen animals that received sodium arsenate, only one did not have cirrhosis; this rabbit died on the third day, and there were necroses in the liver (fig. 1 *A*). The cirrhosis was early in five, moderately advanced in seven and severe in two of the rabbits.

Each of the sixteen animals to which lead arsenate was given had cirrhosis. Three had early, seven moderately advanced and six severe cirrhosis. Thus of the forty-six animals in the series, forty-two (91 per cent) had cirrhosis; of the four without cirrhosis, one died on the third day and another on the sixteenth.

Recent or healed necroses were found in thirty-one livers and necrosis of single liver cells in four. Phagocytes in varying numbers were present in all but five of the forty-six livers, and pigment was increased in thirty-one; all or part of the pigment gave a positive reaction for iron in twenty-nine. Jaundice was observed twice in the animals that received sodium arsenate, three times in those given copper arsenate and four times in those to which lead arsenate was administered. None of the animals had ascites.

Five rabbits were fed on the diet of hay and oats but were not given any of the arsenates. They lived from 193 to 588 days. One rabbit (134) had slight, patchy cirrhosis suggesting healed coccidiosis. In none were there necroses or cirrhosis as described for the animals that received the arsenates. Pigment was increased in four, though the increase was slight; in one of these about half of the pigment was iron containing (table 4).

DIET OF HAY, OATS, CARROTS AND CABBAGE

Eighteen rabbits were fed hay and oats; the diet was supplemented by giving carrots on one day and cabbage the next.

To eight of these animals was given a daily dose of 6.18 mg. of sodium arsenate (1.5 mg. of arsenic). Five received 5.6 mg. of copper arsenate (1.4 mg. of arsenic) each day, and the remaining five, lead arsenate, 7.2 mg. (1.4 mg. of arsenic). The arsenates were administered as described in the foregoing section. The results of this experiment are shown in table 2. The lesions produced were identical with those

TABLE 2.—*Observations on Rabbits Fed Arsenates with a Diet of Oats, Hay Carrots and Cabbage*

| Rabbit | Days | Cirrhosis of Liver | Content of Mineral Element in Liver, Mg. per 100 Gm. of Dry Weight | | | Content of Mineral Element in Kidney, Mg. per 100 Gm. of Dry Weight | | |
|---|---|---|---|---|---|---|---|---|
| | | | Arsenic | Lead | Copper | Arsenic | Lead | Copper |
| | | Rabbits Given Copper Arsenate | | | | | | |
| 109 | 57 | Early | 0.490 | ..... | 1.463 | 0.528 | ..... | 4.488 |
| 108 | 61 | Early | 0.453 | ..... | 0.513 | 0.444 | ..... | 3.696 |
| 110 | 74 | Early | 1.060 | ..... | 2.453 | 1.160 | ..... | 3.380 |
| 107 | 75 | Early | 2.323 | ..... | 3.276 | 4.444 | ..... | 9.556 |
| 106 | 188 | Early | 0.640 | ..... | ..... | 2.440 | ..... | 8.000 |
| | | Rabbits Given Lead Arsenate | | | | | | |
| 117 | 69 | None | 0.770 | 0 | 0.686 | 0.472 | ..... | 7.012 |
| 118 | 104 | Early | 0.830 | 0 | 0.356 | 0.460 | ..... | 3.416 |
| 116 | 230 | Moderately advanced | 3.117 | ..... | 0.440 | 1.928 | ..... | 1.628 |
| 120 | 511 | Early | 4.500 | ..... | ..... | 0.590 | ..... | ..... |
| 119 | 604 | None | 0.067 | 0.583 | ..... | 0.232 | ..... | ..... |
| | | Rabbits Given Sodium Arsenate | | | | | | |
| 126 | 2 | None | 1.550 | ..... | 3.370 | ..... | ..... | ..... |
| 129 | 3 | None | 3.967 | ..... | 1.253 | ..... | ..... | ..... |
| 127 | 23 | Severe | 1.707 | ..... | 1.603 | ..... | ..... | ..... |
| 147 | 38 | Early | 1.010 | ..... | 3.170 | 1.792 | ..... | 7.404 |
| 148 | 39 | Moderately advanced | 1.356 | ..... | 2.553 | 2.132 | ..... | 4.912 |
| 146 | 41 | None | 0.260 | ..... | ..... | 2.284 | ..... | 12.516 |
| 128 | 43 | Severe | 1.207 | ..... | 0.312 | ..... | ..... | ..... |
| 130 | 126 | Moderately advanced | 3.420 | ..... | ..... | 2.064 | ..... | ..... |

found in the animals fed on hay and oats and given the arsenates, but were less severe. One of the animals had ascites; it had received sodium arsenate.

It will be noted that of the eighteen animals, five did not have cirrhosis. Three of these (126, 129 and 146) received sodium arsenate and died on the second, third and forty-first days of the experiment. The other two (117 and 119), given lead arsenate, lived for 69 and 604 days. In none of the five animals were there necroses in the liver.

Each of the five rabbits of the copper arsenate group had cirrhosis, but the lesion was not advanced. Of the three remaining animals of the lead arsenate group, two had early cirrhosis, and in the third the cirrhosis was moderately advanced. Five of the rabbits receiving sodium arsenate acquired cirrhosis; in one the cirrhosis was early, in two moder-

ately advanced and in the other two severe. The cirrhosis was especially striking in one rabbit (127) that died on the twenty-third day. The lobulation of the liver was irregular, and pigment-containing phagocytes were numerous (fig. 2 *C*).

Recent or healed necroses were present in twelve of the thirteen animals in which cirrhosis developed. Phagocytes were found in two of the noncirrhotic livers, and in twelve of the thirteen with cirrhosis. Pigment was increased in the livers of fifteen of the eighteen animals, and in nine the pigment was in part iron containing. The surface of the liver was smooth in twelve rabbits; in the other six it was slightly irregular.

Five rabbits were fed the diet and not given arsenate. Four of these were killed after 589 days. Necroses were not found in any of the livers, nor was there cirrhosis. A slight increase of pigment was noted in one liver; the pigment did not give a reaction for iron (table 4).

### DIET OF WHITE BREAD AND POTATOES

A diet of white bread and peeled white potatoes, uncooked, was fed to twenty-six rabbits. The diet was not especially pleasing to the animals, and a few days were allowed for them to become accustomed to it. The arsenate was sprinkled over the bread and potatoes.

Seven of the rabbits received lead arsenate daily, the dose being 2.4 mg. (0.465 mg. of arsenic); to ten, 1.86 mg. of copper arsenate (0.465 mg. of arsenic) was administered each day. The remaining nine animals were given sodium arsenate. The daily dose through the tenth day was 6.18 mg. (1.5 mg. of arsenic), but as this quantity was not well tolerated, the amount was reduced to 2.06 mg (0.494 mg. of arsenic) (table 3).

Early cirrhosis was found in only two of the animals; one had been given copper arsenate—the other, sodium arsenate. Necroses were not found in the livers of the animals that received sodium or lead arsenate. A few hepatic necroses were discovered in three of the rabbits given copper arsenate, and in another from this group single liver cells had undergone necrosis. Phagocytes were not so numerous as in the other two series of animals. Pigment was increased in the liver in thirteen of the eighteen animals, but the increase was not striking; in three, all of the pigment gave the reaction for iron; in seven, iron-containing pigment was mixed with other pigment that did not give a positive reaction for iron. Considerable glycogen was present in the liver cells. In no animal of this series was the surface of the liver irregular.

The livers of the five control rabbits kept on the same diet did not show either necroses or cirrhosis (table 4).

TABLE 3.—*Observations on Rabbits Fed Arsenates with a Diet of Bread and Potatoes*

| Rabbit | Days | Cirrhosis of Liver | Content of Mineral Element in Liver, Mg. per 100 Gm. of Dry Weight | | |
|---|---|---|---|---|---|
| | | | Arsenic | Lead | Copper |
| | | **Rabbits Given Copper Arsenate** | | | |
| 25 | 11 | None | ..... | ..... | ..... |
| 26 | 23 | None | ..... | ..... | ..... |
| 24 | 44 | None | 7.800 | ..... | 35.000 |
| 29 | 48 | None | 5.800 | ..... | 25.000 |
| 31 | 49 | None | 8.700 | ..... | 18.000 |
| 27 | 59 | None | 2.000 | ..... | 27.000 |
| 22 | 62 | None | 31.000 | ..... | ..... |
| 28 | 96 | None | 0.174 | ..... | ..... |
| 30 | 119 | None | Trace | ..... | 3.370 |
| 23 | 137 | Early | 0.225 | ..... | 2.958 |
| | | **Rabbits Given Lead Arsenate** | | | |
| 46 | 8 | None | ..... | ..... | ..... |
| 42 | 22 | None | ..... | ..... | ..... |
| 48 | 24 | None | 3.400 | 0.700 | ..... |
| 44 | 37 | None | 46.000 | 5.200 | ..... |
| 49 | 41 | None | 2.100 | 1.500 | ..... |
| 45 | 98 | None | 2.607 | 0 | ..... |
| 43 | 119 | None | 0.740 | 1.017 | ..... |
| | | **Rabbits Given Sodium Arsenate** | | | |
| 32 | 2 | None | ..... | ..... | ..... |
| 36 | 13 | None | ..... | ..... | ..... |
| 41 | 21 | None | ..... | ..... | ..... |
| 39 | 25 | None | ..... | ..... | ..... |
| 34 | 58 | None | ..... | ..... | ..... |
| 33 | 62 | None | ..... | ..... | ..... |
| 40 | 119 | Early | 0.907 | ..... | 0.463 |
| 38 | 124 | None | 0.717 | ..... | 2.410 |
| 35 | 128 | None | 0.147 | ..... | 1.883 |

TABLE 4.—*Observations on Controls*

| Rabbit | Days | Cirrhosis of Liver | Content of Mineral Element in Liver, Mg. per 100 Gm. of Dry Weight | | | Content of Mineral Element in Kidney, Mg. per 100 Gm. of Dry Weight | | |
|---|---|---|---|---|---|---|---|---|
| | | | Arsenic | Lead | Copper | Arsenic | Lead | Copper |
| | | **Rabbits Fed Oats and Hay** | | | | | | |
| 135 | 193 | None | 0.213 | ..... | 0.547 | Trace | ..... | 4.748 |
| 134 | 309 | Early | Trace | ..... | 3.100 | 0 | ..... | 1.368 |
| 132 | 500 | None | Trace | ..... | ..... | Trace | ..... | ..... |
| 133 | 519 | None | 0 | ..... | 13.167 | ..... | ..... | ..... |
| 131 | 588 | None | 0.050 | ..... | 3.020 | Trace | ..... | ..... |
| | | **Rabbits Fed Oats, Hay, Carrots and Cabbage** | | | | | | |
| 138 | 406 | None | 0 | ..... | 1.067 | 0 | ..... | 0 |
| 136 | 589 | None | 0 | ..... | 1.527 | 0 | ..... | 0 |
| 137 | 589 | None | 0 | ..... | 0.901 | 0 | ..... | 0 |
| 139 | 589 | None | 0 | ..... | 1.427 | 0 | ..... | 0 |
| 140 | 589 | None | 0 | ..... | 0.920 | 0 | ..... | 0 |
| | | **Rabbits Fed White Bread and Potatoes** | | | | | | |
| 170 | 67 | None | ..... | ..... | 4.500 | ..... | ..... | ..... |
| 174 | 70 | None | ..... | ..... | 8.100 | ..... | ..... | ..... |
| 168 | 147 | None | ..... | ..... | 1.000 | ..... | ..... | ..... |
| 194 | 152 | None | ..... | ..... | 2.750 | ..... | ..... | ..... |
| 198 | 154 | None | ..... | ..... | ..... | ..... | ..... | ..... |

### CHEMICAL ANALYSES

Numerous livers and kidneys were analyzed to determine the quantities of arsenic, copper and lead present. The amounts are given in milligrams per hundred grams of the dried organ (tables 1, 2, 3 and 4).

*Diet of Hay and Oats and Diet of Hay, Oats, Carrots and Cabbage.*—The amount of arsenic in the liver was determined in forty-five of the forty-six rabbits on the diet of hay and oats and in the eighteen that were fed hay, oats, carrots and cabbage; each of these sixty-four rabbits was given one of the arsenates. The amount of arsenic found varied from 0.067 to 20.420 mg., the average being 2.985 mg. It is apparent from the tables that there is no constant relationship between the amount of arsenic present in the liver and the severity of the cirrhosis; neither is the arsenic content proportional to the length of time the animal lived. The kidneys from forty-five of these sixty-four rabbits were analyzed for arsenic. The quantity found was from 0.232 to 18.640 mg. In many instances the quantity of arsenic in the kidney was proportional to that in the liver. There were fifty-seven determinations of the copper content of the liver, and it was found to vary from 0.312 to 8.900 mg., the average being 1.914 mg. The amount of copper was somewhat greater among the animals that received the copper arsenate. From 1.628 to 12.128 mg. of copper were found in the kidneys; thirty-six determinations were made.

Seventeen analyses were made for the lead content of the liver; fifteen of the animals had received lead arsenate; the other two, sodium arsenate. Lead was not found in thirteen; in two there were traces, and two showed 0.583 and 3.643 mg. Twelve of the thirteen livers in which no lead was found were from animals that had been given lead arsenate.

*Diet of White Bread and Potatoes.*—Analyses for arsenic were made of sixteen livers from the series of twenty-six rabbits fed on white bread and potatoes and given the arsenates. It will be recalled that these animals received smaller doses of arsenates than those on the other two diets. A trace of arsenic was found in one, and in the other fifteen the amounts varied from 0.147 to 46.00 mg., with an average of 7.489 mg. The largest amounts of arsenic found in any livers from the rabbits used in the entire experiment were in this series—31 and 46 mg. One of these animals received copper arsenate; the other, lead arsenate. Analyses for copper content were made on the livers of nine rabbits: three animals that received sodium arsenate and six from among those given copper arsenate. The livers of the rabbits given sodium arsenate contained amounts of copper comparable to those found in the other two series. But this similarity does not obtain in the copper arsenate group. In four of the six livers the amounts of copper—18, 25, 27

and 35 mg.—were several times greater than the largest amount found in the livers of the corresponding groups of rabbits on the other diets. The average copper content of the liver as determined in the nine livers analyzed was 12.897 mg.

Five of the livers of the animals that received lead arsenate were analyzed for lead. In only one of these was lead not found; the other four contained from 1.017 to 6.70 mg. This is in striking contrast to the negative results from the analyses of twelve livers from fifteen animals fed on the other diets and given lead arsenate.

*Controls.*—The livers of the control rabbits fed hay and oats or hay, oats, carrots and cabbage were analyzed for arsenic. In six no arsenic was found; in two there were traces, and in two, 0.050 and 0.213 mg. The four animals in whose livers some arsenic was found had been fed hay and oats. The copper content of the liver was determined in thirteen of the control animals. The amounts present varied from 0.547 to 13.167 mg. No analyses for lead were made.

### RATS AND FERRETS

Fifty-one rats were divided into three groups. One group of sixteen received lead arsenate; another of like number were given copper arsenate, and 19, sodium arsenate. Many of these animals lived from 250 to over 500 days after the experiment was begun. In no instance were necroses found in the liver, nor was there cirrhosis.

Fifteen ferrets were divided into groups of five. To each group of animals was fed one of the arsenates daily. None of the animals that received lead arsenate lived longer than 48 days. One ferret given copper arsenate died on the thirty-first day; the other four lived from 120 to 329 days. In the sodium arsenate group, one animal died at the end of a week; two lived 60 days, and the other two, 216 and 266 days. In none of these fifteen animals was there cirrhosis or any necrosis in the liver.

### COMMENT

Lead and copper arsenates, used in these experiments, are frequently employed as insecticides on fruit trees and vegetables. For use as lead arsenate, one of the commercial products sold for this purpose was purchased in the open market. This product is 98 per cent lead arsenate. Sodium arsenate was selected to control any possible effect of the lead and copper in the other two arsenates. Oral administration was employed, as this is the manner in which arsenates contaminating food products gain entrance into the body. Sturmer [9] has estimated that 80,000,000 pounds of arsenical compounds are used annually on fields,

9. Sturmer, J. W.: Am. J. Pharm. **104**:758, 1932.

orchards and lawns in the United States. The diet of rabbits usually consists in part of those vegetables that are commonly sprayed or dusted with these insecticides. In view of the results reported here, care should be exercised that such arsenical compounds are not present in the diet of rabbits used experimentally to test various substances for their possible cirrhogenic properties.

A biopsy of the liver was done on every rabbit employed in the experiment with one exception. In each specimen of liver so removed, pigment was found to be present before the experiment was begun, and in some the amount was large. The exposure of the liver afforded an opportunity to examine it for coccidiosis before the experiment was undertaken.

The experiments emphasize the importance of diets. Of the forty-six rabbits fed hay and oats and given a daily dose of one of the arsenates only four—9 per cent—failed to acquire cirrhosis. Two of these four lived only a short time. That the arsenate did, however, produce damage in the livers of three of these four is indicated by the presence of necroses. With this diet, lead arsenate was most effective in producing cirrhosis.

When the diet of hay and oats was supplemented by the addition of carrots and cabbage, the incidence and severity of the cirrhosis were decreased. An even more striking effect of the protection afforded by diet is seen in the rabbits fed on white bread and potatoes. In this series of twenty-six rabbits, only two presented cirrhosis, which was of a mild type; the other twenty-four, or 91 per cent, did not have any increase of connective tissue in the liver. These divergent results emphasize the necessity of recording the diets in reports of experiments on cirrhosis when rabbits have been used.

The chief difference in these diets appears to be in the amounts of carbohydrate present. From this experiment it is evident that the damage produced in the liver by the arsenates was greatest when the diet contained the least amount of carbohydrate. Hooper, Kolls and Wright [10] found that rats fed on white bread, rolled oats and milk were more resistant to the effect of arsphenamine and allied compounds than were those given lean beef heart. They noted, however, that feeding 40 per cent saccharose solution lowered the resistance of the animals. It has long been recognized by clinicians that carbohydrate affords some protection to the liver; those interested in the subject from this standpoint are referred to the review by Althausen.[11]

The analyses for the arsenic contents of the livers of the rabbits that received the arsenates show clearly that the extent of the damage

10. Hooper, C. W.; Kolls, A. C., and Wright, K. D.: J. Pharmacol. & Exper. Therap. **18**:133, 1921.

11. Althausen, T. L.: J. A. M. A. **100**:1163, 1933.

done is not directly related to the amount of arsenic present. The largest average quantities of lead, copper and arsenic were found in those animals that were fed bread and potatoes, and in this series the incidence of cirrhosis was only 9 per cent. There appears to be some relationship between the carbohydrate in the diet and the quantity of the metals found in the liver, but no suggestion can be made at this time as to what this relationship may be, nor as to the way in which carbohydrate protects the liver. The histologic appearance of the liver cells indicated that more glycogen was present in these cells in the animals that ate white bread and potatoes.

The position and character of the necroses found in the livers of the rabbits are similar to those described by the investigators whose reports have been reviewed. However, the acute inflammatory reaction about the necroses was not so marked as that described by Wolkow.[8] No differences could be detected in the character of the lesions produced by the different arsenates. It is therefore concluded that the arsenic alone was responsible and that the lead and copper did not play any part in the production of these lesions.

Healing of the necroses leading to scarring could be readily followed. Since most of the necroses were situated adjacent to the portal areas, the scarring resulted in widening of these structures. Also the presence of isolated groups of liver cells in the widened portal areas can be easily explained. Groups of intact liver cells were frequently observed separated from the remainder of the lobule by necroses. With the healing of the necroses and the outgrowth of connective tissue from the portal area, islands of intact liver cells were permanently cut off from the lobule. It is in this manner that liver cells became incorporated in the widened portal area. The phagocytes are believed to be a response to the presence of necrotic liver cells and are not specific for the damage done to the liver by arsenic. The pigment in the liver was increased in fifty-nine of the ninety rabbits given the arsenates, and the increase was most marked in those rabbits that were not on the diet of bread and potatoes. In seven of the fifteen control animals the pigment in the liver was increased, but not markedly so. In no instance was the pigment increased in control rabbits on the diet of bread and potatoes. The amount of the pigment did not have a constant relation to the severity of the cirrhosis.

It is perhaps necessary to discuss briefly how the cirrhosis produced in the rabbits was classified. When the outline of the lobule was distorted and the lobules were of irregular size, the cirrhosis was classified as severe; when the connective tissue passed from one portal area to another, thus accentuating the lobular pattern, the cirrhosis was designated as moderately advanced; the cirrhoses not fitting into these two categories were listed as early. According to this classifica-

tion the cirrhosis was severe in thirteen, moderately advanced in twenty-four and early in eighteen of the sixty-four rabbits that received the diet of hay and oats with or without the addition of carrots and cabbage. The type of the cirrhosis produced was very different from that due to coccidiosis, and no difficulty was experienced in separating them. The gross appearance of the liver was frequently altered, varying from finely granular to coarsely pebbled. The irregularity of the surface was not the result of regeneration of liver tissue, but was due to the sinking in of the capsule that followed removal of necrotic cells in the necroses and to the scarring.

These experiments also show an individual variation in the susceptibility of rabbits to arsenates. But the failure to produce cirrhosis in the same percentage in rabbits on the diet of bread and potatoes as in those on the other diets cannot be accounted for on the basis of a variation in susceptibility. It is illogical to assume that only highly resistant rabbits happened to be selected for the bread and potato diet.

The negative results with rats and ferrets indicate that so far as damage to the liver is concerned these animals are more resistant to the effects of the arsenates than are rabbits.

From clinical observation there is evidence that arsenic produces cirrhosis in man. Geyer,[12] in reporting chronic arsenical poisoning in Reichenstein in Schlesien, recorded a case in which frequent paracentesis was required. Hamburger [13] reported a case of poisoning from a solution of potassium arsenite (Fowler's solution); the patient had ascites. Sturrock,[14] in discussing "beer poisoning" due to arsenic, mentioned eight cases in which this was associated with ascites and an enlarged, firm liver. Following rest in bed, the ascites rapidly disappeared and the liver decreased in size. Reynolds,[15] in his report of this outbreak, stated that there was an "unusual number of cases of cirrhosis" with ascites. A case of cirrhosis with ascites that followed the taking of arsenic in a bromide mixture was placed on record by Stockman.[16] O'Leary, Snell and Bannick [17] cited two cases in which cirrhosis occurred in chronic arsenic poisoning, and O'Leary, Green and Rowntree [18] mentioned a case in which cirrhosis was believed to have been due to arsphenamine. Weir [19] recorded two cases in which cirrhosis

12. Geyer, L.: Arch. f. Dermat. u. Syph. **43**:221, 1898.
13. Hamburger, L. P.: Bull. Johns Hopkins Hosp. **11**:87, 1900.
14. Sturrock, A. C.: Brit. M. J. **2**:1815, 1900.
15. Reynolds, E. S.: Lancet **1**:166, 1901.
16. Stockman, R.: Edinburgh M. J. **27**:1, 1921.
17. O'Leary, P. A.; Snell, A. M., and Bannick, E. G.: J. A. M. A. **90**:1856, 1928.
18. O'Leary, P. A.; Greene, C. H., and Rowntree, L. G.: Arch. Int. Med. **44**:155, 1929.
19. Weir, J. F.: Proc. Staff Meet., Mayo Clin. **5**:173, 1930.

was ascribed to chronic arsenic poisoning. Dörle and Ziegler [20] reported twenty-five cases in which chronic arsenic poisoning resulted from the application of an arsenical powder to vines. In two of these cases the liver was enlarged and hard; both patients also drank moderate amounts of wine. Rolleston and McNee [21] expressed the opinion that arsenic appears capable of setting up cirrhosis. Twelve cases of cirrhosis among thirty-six persons who had antisyphilitic treatment were described by Baldridge.[22] One of these was observed in a woman who did not use alcohol and did not have syphilis but who requested antisyphilitic therapy. In a recent report of chronic arsenical poisoning, Cannon [23] cited the case of a young woman whose liver was considerably enlarged. The poisoning resulted from ingestion of the arsenical spray used on fruits and vegetables.

## CONCLUSIONS

Lead arsenate, cupric arsenate and sodium arsenate in the doses administered in the experiments reported here cause similar necroses in the livers of rabbits. The healing of these necroses results in cirrhosis.

In the rabbit, a diet rich in carbohydrate protects the liver from the injurious effect of the arsenates.

The lead or copper in the arsenates used in the experiments described did not cause the lesions described.

These arsenates are less injurious to the liver of the rat and ferret than to that of the rabbit.

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20. Dörle, M., and Ziegler, K.: Ztschr. f. klin. Med. **112**:237, 1929.

21. Rolleston, H. D., and McNee, J. W.: Diseases of the Liver, Gall Bladder and Bile Ducts, ed. 3, London, The Macmillan Company, 1929, p. 215.

22. Baldridge, C. W.: Am. J. M. Sc. **188**:685, 1934.

23. Cannon, A. B.: New York State J. Med. **36**:219, 1936.

# EFFECT OF 1,2,5,6-DIBENZANTHRACENE ON SPINDLE CELL SARCOMA OF A RAT

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Two reports on the action of carcinogenic hydrocarbons on malignant growths have appeared. In both adverse effects on the tumors were described. Haddow,[1] the first to report, observed that the Jensen sarcoma grew slowly in rats that were receiving large intraperitoneal doses of carcinogenic hydrocarbons. Although the tumor employed by Haddow is known to regress at times spontaneously, he saw no complete regression during the relatively short period that his animals were under observation, but the rate of growth was much slowed. The second report, which appeared while our own experiments were in progress, was made by Pybus and Miller.[2] These workers injected intraperitoneally a colloidal solution of 1,2,5,6-dibenzanthracene into mice with spontaneous cancers of the mammary glands. They stated that of sixty mice that received injections, the tumors partly or completely regressed in 8, and that in 3 of these the tumors did not reappear before death. The formation of new cancers during treatment was not prevented. The treatment was without effect on sarcoma and leukemia. Although the number of complete regressions was small, it seems that great significance may be attached to the few that occurred, since the cancers do not disappear spontaneously. In both reports, in discussion of the adverse effects on the tumors, mention was made of the apparent reversible action of x-rays and radium in the causation and destruction of blastomatous growth for the purpose of drawing attention to the apparent reversible action of the carcinogenic substances.

Our experiments were continued after the appearance of these publications because it appeared that additional data were being acquired. The tumor with which our experiments were made was a sarcoma which arose spontaneously in the uterus of an individual of our albino rat colony and which during a four year period has been transplanted successfully into more than 1,500 albino rats of the Wistar Institute strain.

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1. Haddow, A.: Nature, London **136**:868, 1935.
2. Pybus, F. C., and Miller, E. W.: Brit. J. Exper. Path. **18**:126, 1937.

The sarcoma has not been seen to regress spontaneously. Subjecting it to several experimental treatments that have caused other types of tumors to regress, we have obtained complete regression only under two conditions, one of which is described in this paper. The other [3] was that the rats inoculated had been thyroparathyroidectomized when about 1 month old. Inoculation with the sarcoma was made about three months after the operation.

### ATTEMPTS TO CAUSE REGRESSION OF TUMORS BY INTRATUMORAL INJECTIONS OF METHYLTHIONINE CHLORIDE U. S. P., SODIUM OLEATE AND CYSTEINE MONOHYDROCHLORIDE

In addition to receiving injections of 1,2,5,6-dibenzanthracene, our sarcoma has received injections of substances which in the hands of others have caused tumors to disappear. A brief statement of some of the results of these other investigators emphasizes the refractory behavior of our sarcoma to substances that caused other types of tumor to disappear. Brooks [4] was able to accomplish regression by daily intratumoral injection of 0.1 per cent aqueous solution of methylthionine chloride. The type of tumor was designated "carcinoma-sarcoma no. 256/94A." Nakamura [5] injected methylthionine chloride in the strength used by Brooks into sarcomas of three types every second day for from one to seven weeks. The injection had only a slight inhibitory effect. We injected methylthionine chloride (Gruebler) intratumorally into twenty-one rats, in both 0.01 and 1 per cent strengths, with little or no effect on the rate of tumor growth (table 1). When the stronger solution was injected into rats considerably younger than those listed in the table, extensive ulceration and sloughing followed but the tumor continued to extend at its periphery.

Begg and Aitken [6] were able to cause disappearance of the Rous chicken sarcoma by injecting intratumorally 1 per cent sodium oleate neutralized with hydrochloric acid. After the regression was complete, reinoculation failed. The regression was associated with a definite increase in the lipolytic power of the serum. We were unable to cause regression of our sarcoma by injecting sodium oleate (table 1).

Connor, Carr and Ginzton [7] accomplished complete regression of the Jensen sarcoma with intratumoral injections of cysteine hydrochloride. We injected into our sarcoma cysteine monohydrochloride.

3. McJunkin, F. A.; Templeton, R. D., and Kravec, F. G.: Proc. Soc. Exper. Biol. & Med. **34**:801, 1936.

4. Brooks, M. M.: Proc. Soc. Exper. Biol. & Med. **30**:1001, 1933.

5. Nakamura, H.: Tr. Soc. path. jap. **25**:721, 1935.

6. Begg, A. M., and Aitken, H. A. A.: Brit. J. Exper. Path. **13**:479, 1932.

7. Connor, C. L.; Carr, J. L., and Ginzton, L.: Proc. Soc. Exper. Biol. & Med. **34**:374, 1936.

Extensive ulceration followed, with sloughing of the central necrotic portion. The periphery, however, continued to grow rapidly, and regression was not accomplished.

### EXPERIMENTS WITH 1,2,5,6-DIBENZANTHRACENE[8]

This carcinogenic hydrocarbon may be effectively emulsified in aqueous form by mixing it with lecithin in a mortar and adding water.

TABLE 1.—*Intratumoral Injections of Methylthionine Chloride, Sodium Oleate and Cysteine*

| Litter Number | Weight When Given Injection (Average), Gm. | Injection | Duration of Experiment From Time of First Injection (Average), Days | Average Diameter of Tumors, Cm. | |
|---|---|---|---|---|---|
| | | | | At Beginning of Experiment | At Conclusion |
| A | | | | | |
| 1,2,3,4,5 i* | 134 | 1.2 cc. of 0.01% methylthionine chloride (12 ×)† | | 1.6 i | 3.2 i |
| 6 c* | | | 17 | 1.6 c | 3.4 c |
| B | | | | | |
| 1,2,3,4,5 i | 119 | 1.2 cc. of 0.01% methylthionine chloride (14 ×) | 17 | 1.4 i | 2.3 i |
| 6 c | | | | 1.4 c | 2.5 c |
| C | | | | | |
| 1,2,3,4,5,6 i | 125 | 1.2 cc. of 0.01% methylthionine chloride (12 ×) | 16 | 1.5 i | 3.2 i |
| 7 c | | | | 1.6 c | 3.5 c |
| D | | | | | |
| 1,2,3,4,5 i | 135 | 1.2 cc. of 1% methylthionine chloride (3 ×) | 27 | 2.0 i | 5.3 i |
| 6 c | | | | 1.8 c | 5.2 c |
| E | | | | | |
| 1,2,3 i | 123 | 1 cc. of 1% sodium oleate neutralized with | 30 | 1.5 i | 3.2 i |
| 4 c | | hydrochloric acid (4 ×) | | 1.2 c | 2.9 c |
| F | | | | | |
| 1,2,3,4 i | 96 | 1 cc. of 1% sodium oleate neutralized with | 16 | 1.2 i | 2.4 i |
| 5 c | | hydrochloric acid (3 ×) | | 1.3 c | 2.6 c |
| G | | | | | |
| 1,2,3,4,5 i | 113 | 1 cc. of 1% sodium oleate neutralized with | 12 | 1.4 i | 2.1 i |
| 6 c | | hydrochloric acid (4 ×) | | 1.2 c | 2.4 c |
| H | | | | | |
| 1,2,3,4 i | 109 | 1 cc. of distilled water containing 25 mg. of | 9 | 1.9 i | 2.5 i |
| 5 c | | cysteine monohydrochloride (1 ×) | | 1.9 c | 2.6 c |
| I | | | | | |
| 1,2,3,4,5 i | 82 | 1 cc. of distilled water containing 25 mg. of | 13 | 1.4 i | 2.9 i |
| | | cysteine monohydrochloride (2 ×) | | 1.4 c | 3.0 c |
| J | | | | | |
| 1,2,3,4,5,6 i | 121 | 1 cc. of distilled water containing 25 mg. of | 9 | 1.8 i | 2.8 i |
| 7 c | | cysteine monohydrochloride (2 ×) | | 1.8 c | 3.0 c |

* The rats given injections are designated by i; the control rats (not given injections) by c.
† The injection of 1.2 cc. of the aqueous solution was repeated twelve times.

Such an emulsion is more readily absorbed than the oil solution and the aqueous suspensions that we have tried. In older rats the peritoneal irritation is less pronounced, but in young rats extensive ascites follows administration of large doses. Not only do older rats withstand the treatment better, but in these it is easier to accomplish complete regression of the tumors. Twelve sets of experiments in which both young

8. The 1,2,5,6-dibenzanthracene was purchased from the Eastman Kodak Company, Rochester, N. Y.

and old rats were used are included in table 3. In all animals at the time of the first injection the tumor measured 1 cm. or more in diameter. Of fifty-one tumor-bearing rats that received injections of the dibenzanthracene-lecithin emulsion, the tumors completely disappeared in twenty. In many of the others the injections caused profound slowing of tumor growth. In table 3 appear the average diameters of the tumors at the time of the death of the animals or the termination of the experiment. The courses taken by the individual members of the sets reveal more accurately the effects of the treatment. The descriptions follow.

TABLE 2.—*Intratumoral Injections of 1,2,5,6-Dibenzanthracene and Lecithin*

| Litter Number | Weight When Given Injection (Average), Gm. | Injection | Duration of Experiment From Time of First Injection (Average), Days | Average Diameter of Tumors, Cm. | |
|---|---|---|---|---|---|
| | | | | Beginning of Experiment | Conclusion of Experiment |
| A | | | | | |
| 1,2,3,4,5 i* 6 c* | 138 | 1 cc. of aqueous emulsion (1 cc. containing 10 mg. of 1,2,5,6-dibenzanthracene and 142.8 mg. of lecithin [Eastman Practical]) at weekly intervals (4 ×) | 36 | 1.8 i 1.5 c | 2.2 i 6.3 c |
| B | | | | | |
| 1,2,3,4 i 5 c | 144 | 1 cc. of aqueous emulsion (1 cc. containing 10 mg. of 1,2,5,6-dibenzanthracene and 142.8 mg. of lecithin [Eastman Practical]) at weekly intervals (3 ×) | 29 | 2.0 i 1.3 c | 2.6 i 5.8 c |
| C | | | | | |
| 1,2,3,4,5,6,7,8 i 9,10 c | 128 † | 1 cc. of aqueous emulsion (1 cc. containing 10 mg. of 1,2,5,6-dibenzanthracene and 142.8 mg. of lecithin [Eastman Practical]) at weekly intervals (3 ×) | 28 | 1.3 i 1.3 c | 3.0 i 5.3 c |
| D | | | | | |
| 1,2,3,4,5,6,7 i 8,9 c | 110 † | 1 cc. of aqueous emulsion (1 cc. containing 10 mg. of 1,2,5,6-dibenzanthracene and 142.8 mg. of lecithin [Eastman Practical]) at weekly intervals (3 ×) | 35 | 1.4 i 1.4 c | 3.2 i 6.2 c |
| E | | | | | |
| 1,2,3,4,5 i 6 c | 113 | 1 cc. of aqueous emulsion (1 cc. containing 142.8 mg. of lecithin only) at weekly intervals (3 ×) | 26 | 1.9 i 2.0 c | 5.2 i 5.6 c |
| F | | | | | |
| 1,2,3,4,5,6 i 7,8 c | 112 | 1 cc. of aqueous emulsion (1 cc. containing 142.8 mg. of lecithin only) at weekly intervals (3 ×) | 31 | 1.3 i 1.4 c | 5.5 i 5.7 c |

* The rats given injections are designated by i; the controls by c.
† These were not litter mates.

A. One of the four rats given injections died on the twenty-first day with ascites. The other three showed extensive ascites when killed. In all of them microscopic examination of the tumor remnants showed extensive necrosis and fibrosis with scattered peripheral groups of living tumor cells.

B. Two of the rats given injections died on the tenth day with ascites. These and two others showed foci of living tumor cells on microscopic examination. The fifth rat showed no palpable tumor on the eighth day. On the twelfth day the tissues of the groin at the site of the regressed tumor showed no tumor cells on thorough microscopic examination.

C. Of the five rats given injections, four showed on the twelfth day complete regression of their tumors, which at the time of injection measured 1.2, 1.2, 1.2 and 1 cm., respectively. In the fifth rat the tumor had increased 0.3 cm. in diameter when the experiment was terminated.

TABLE 3.—*Intraperitoneal Injections of 1,2,5,6-Dibenzanthracene and Lecithin*

| Litter Number | Average Weight or Age When Given Injection | Injection of Emulsion‡ | Duration of Experiment From Time of First Injection (Average), Days | Average Diameter of Tumor, Cm. | |
|---|---|---|---|---|---|
| | | | | Beginning of Experiment | Conclusion of Experiment§ |
| A | | | | | |
| 1,2,3,4 i* | 50 days | 28.5 mg. dba and 28.5 mg. lecithin (7 ×) | 33 | 1.2 i | 1.9 i |
| 5,6 c* | | | | 1.1 c | 6.0 c |
| B | | | | | |
| 1,2,3,4,5 i | 91 Gm. | 66.6 mg. dba and 66.6 mg. lecithin (3 ×) | 12 | 1.1 i | 1.5 i |
| 6,7 c | 53 days† | | | 1.0 c | 2.8 c# |
| C | | | | | |
| 1,2,3,4,5 i | 171 days | 100 mg. dba and 1 Gm. lecithin (1 ×) | 12 | 1.2 | See text |
| 6,7,8 c | | | | 1.2 | 2.1 c |
| D | | | | | |
| 1,2,3,4 i | 70 days | 30 mg. dba and 300 mg. lecithin (1 ×) | 15 | 1.3 i | 1.9 i |
| 5,6,7 c | † | | | 1.7 c | 4.0 c |
| E | | | | | |
| 1,2,3,4,5 i | 46 days | 20 mg. dba and 300 mg. lecithin (3 ×) | 33 | 1.4 i | 1.4 i |
| 6,7 c | | | | 1.1 c | 2.8 c |
| F | | | | | |
| 1,2,3,4,5 i | 251 Gm. | 20 mg. dba and 200 mg. lecithin (3 ×) | 15 | 1.2 i | See text |
| 6 c | † | | | 1.2 c | 2.7 c |
| G | | | | | |
| 1,2,3,4,5 i | 226 Gm. | 20 mg. dba and 200 mg. lecithin (2 ×) | 13 | 1.1 i | See text |
| 6 c | † | | | 1.0 c | 2.6 c |
| H | | | | | |
| 1,2,3,4 i | 298 Gm. | 20 mg. dba and 200 mg. lecithin (1 ×) | 23 | 1.4 i | See text |
| 6 c | † | | | 1.2 c | |
| I | | | | | |
| 1,2,3,4 i | 276 Gm. | 20 mg. dba and 200 mg. lecithin (1 ×) | 23 | 1.3 i | See text |
| 5 c | † | | | 1.3 c | |
| J | | | | | |
| 1,2,3,4,5 i | 140 Gm. | Lecithin only (see text) | 12 | 1.5 i | 2.8 i |
| 6,7,8 c | | | | 1.8 c | 2.9 c |
| K | | | | | |
| 1,2,3,4,5 i | 278 Gm. | 20 mg. dba and 200 mg. lecithin (1 ×) | 22 | 1.2 i | See text |
| 6,7,8 c | † | | | 1.1 c | |
| L | | | | | |
| 1,2,3,4,5 i | 288 Gm. | 20 mg. dba and 200 mg. lecithin (1 ×) | 22 | 1.2 i | |
| 6,7 c | † | | | 1.2 c | |

* The rats given injections are designated by i; the controls by c.
† These rats were not litter mates but were rats of about the same age or weight.
‡ This was an aqueous emulsion of 1,2,5,6-dibenzanthracene (dba) and lecithin.
§ See text for description of final results.
# The controls received lecithin in amounts equal to those of the lecithin given the dibenzanthracene-lecithin treated rats.

D. One of the four rats given injections showed complete regression on the fifteenth day. Of the tumors in the three others, one increased 0.3 cm., another 0.4 cm. and the third 1 cm. in size.

E. Two of the five young rats that received injections were dead on the fifteenth day. The tumors of these rats showed no increase in size. Of the tumors in the other three, two increased 0.5 cm. each and the third 0.6 cm.

F. Three of the five rats died nine days after the experiment began. Of these three rats at the time of death, one showed a decrease in the size of the tumor from 1.5 to 1 cm. In the other two, the tumor remained stationary. The two that lived had complete regression of the tumor. The injections were made on the first, third and seventh days.

G. Three died before conclusion of the experiment with no increase in the size of their tumors. Of the two that lived, one showed complete regression of its tumor, and in the other the tumor remained stationary. The injections were made on the first and sixth days.

H. Of the four rats given injections, the tumors regressed completely in three while in the fourth the tumor increased 0.7 cm. in diameter. The control died with a large tumor.

I. Of the four animals given injections, one died, and no record was made of it. On the twenty-third day two showed complete regression from 1.2 cm. to 0. In the other the tumor had increased to 2.5 cm. The tumor of the control measured 3.3 cm.

J. Rats 1, 2, 3, 4 and 5 received on the first and third days 200 mg. of lecithin. Rats 6, 7 and 8 received no treatment whatever.

K. Of the five rats that received injections, the tumors of two regressed from 1 and 1.2 cm., respectively, to 0 in twelve days. In a third the tumor disappeared in twenty-two days. A fourth had a tumor measuring 1.9 cm. on the twenty-second day, and a fifth died with a tumor measuring 1.2 cm. Of the controls, one died with a large tumor (unmeasured). In the other two controls the tumors measured 4.8 cm. and 4 cm., respectively.

L. Of five rats given injections, three showed complete regression of the tumors on the twelfth days. A fourth died, and no record was made. A fifth had a tumor that measured 1.9 cm. on the twenty-second day. One control died with a large tumor, and the other had a tumor that measured 4.1 cm.

## COMMENT

Evidence has been presented to show that a nonregressing type of sarcoma can be caused to disappear by intraperitoneal injections of emulsions of one of the powerful carcinogenic coal tar derivatives. Although the transplanted tumor has become well established and has reached a considerable size when the injections are initiated, the sarcoma undergoes complete regression by processes of necrosis and fibrosis. The accomplishment is rather easy in older rats. In table 3 there is included only one group (set J) of rats treated with lecithin only. Lecithin alone has little effect on the growth of this tumor. However, in another series of experiments not included in this report, many rats were given intraperitoneal and subcutaneous injections of emulsified lecithin, with no

complete regression observed and with little inhibition of the tumor growth. Obviously it is the dibenzanthracene that determines the regression of the tumor. Of twenty-nine rats (table 2) that received intratumoral injections of the 1,2,5,6-dibenzanthracene-lecithin emulsion, none showed complete regression. Although the amounts injected are not exactly comparable, it is evident that this method of administration is much less effective in arresting tumor growth than that of intraperitoneal injection. We were led to use the intratumoral injections because injections of methylthionine chloride, sodium oleate and cysteine hydrochloride had, in the hands of others, caused malignant growths to regress and disappear. These three substances failed to cause complete regressions of our sarcoma (table 1).

An explanation of the regressions brought about by the injections of dibenzanthracene-lecithin emulsions based on established facts is not at hand. McJunkin, Hemwall and Fullgrabe[9] made experiments showing that serum butyrase may be lowered by intraperitoneal injections of cholesterol. In unpublished experiments it was determined that injections of 1,2,-5,6-dibenzanthracene similarly lowered the power of the serum to split ethyl butyrate, but we have no proof that dibenzanthracene acts to inhibit growth of sarcoma by influencing enzyme action. The carcinogenic substances derived from crude high boiling point tar, first used in the production of experimental cancers, cause destructive local effects in the tissues and definitely are not stimulators of primary growth. This is true also for the purified potent compounds, such as 1,2,5,6-dibenzanthracene, now available. Under the influence of the agent the tissue cell, whether of connective tissue or of epithelium, adapts itself to survival under a localized specific adverse condition; its emergence as a neoplastic cell, resistant to the tissue inhibitors, may result. It may well be that the highly potent carcinogenic agents have the strongest property of stimulating inhibitors of growth and that, over a period of months, a new cell able to resist the inhibition would arise. The cellular transformation would be strictly localized in the area about the irritant. Now might not an enormous general production of factors that slow growth follow the quick introduction of carcinogenic substances into the blood and tissues—for example, by intraperitoneal injection? It is noteworthy that direct injection of the chemical into the tumor is less effective than intraperitoneal administration. Might not such a mechanism explain the inhibition exerted on localized neoplasms by such injections? Additional data are required to answer the questions. Franks and Creech,[10] of the Banting Institute, have been reported to have injected

9. McJunkin, F. A.; Hemwall, G. A., and Fullgrabe, E. A.: Am. J. Path. **12**:748, 1936.

10. Franks, W. R., and Creech, J. J.: Science **85**:10, 1937.

coal tar derivatives combined with various proteins into animals with the result that there appeared in the serum antibodies which had the property of uniting with the cancer-producing chemical.

SUMMARY

A spindle cell sarcoma that does not regress spontaneously in the normal rat can be made to disappear by intraperitoneal injections of aqueous emulsions of 1,2,5,6-dibenzanthracene and lecithin. A possible explanation of the observed regression has been discussed.

# FATE OF ERYTHROCYTES AND GRANULOCYTES IN THE SPLEEN FOLLOWING THEIR INJECTION INTO THE BLOOD STREAM

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When erythrocytes foreign to a species are introduced into the blood stream, they are ingested by phagocytes in the spleen and other organs. The present study was undertaken to determine the fate of red blood corpuscles of the same species injected into the blood, in order to learn how the body gets rid of its own blood corpuscles when these become effete. Homologous washed blood corpuscles in considerable amount have been injected into the blood stream, and their fate in the spleen has been studied. The study has included both red and white corpuscles and has given an excellent opportunity to study the phagocytosis of these corpuscles in detail.

The fate of red corpuscles of the hen when these were injected intravenously into the rabbit was studied by Gowan.[1] That hemolysis occurred was shown by the appearance of hemoglobinemia and hemoglobinuria shortly after the injection. The avian corpuscles disappeared from the circulation within thirty minutes and were found accumulated in the capillaries of the liver. Gowan stated that the serum of the normal rabbit was more or less hemolytic for corpuscles of the hen and that when hemolysis was conspicuous in vitro it proceeded rapidly in vivo, and that there was no phagocytosis of avian erythrocytes by the Kupffer cells of the liver or by phagocytes of the spleen. Addison [2] injected washed corpuscles of the pigeon into the blood stream of the rabbit, perfused the spleen with salt solution after different intervals following the injection and examined the organs histologically. The avian erythrocytes accumulated in the spleen and there underwent changes, the nuclei no longer staining and the cell bodies breaking into round and irregular fragments. Whole corpuscles and fragments often were engulfed by large cells free in the sinuses of the pulp ("splenocytes"). In the same cells was found a yellow-brown pigment that gave microchemical reactions of iron. There was hemoglobinuria for twelve hours after the injection. Phagocytes were largest about sixteen hours after the injection, and after forty-eight hours they were approximately normal in size. Polymorphonuclear leukocytes and myelocytes of the rabbit accumulated in the spleen after the injection of avian corpuscles, and many of them were ingested by phagocytes.

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1. Gowan, J. P.: J. Path. & Bact. **14**:379, 1910.
2. Addison, W. H. F.: Am. J. Anat. **26**:437, 1920.

Corpuscles of the ox, washed free from serum, were injected by Cary [3] into the veins of rabbits. After a single injection there was phagocytosis of erythrocytes in the spleen of the recipient. Three injections produced greater phagocytosis in the spleen and some in the liver. After from four to ten injections there was abundant phagocytosis in both the spleen and the liver.

Transfusion of whole blood into the blood stream of an animal of the same species induces artificial destruction of blood. Hunter [4] found that transfusion of blood into rabbits is followed by accumulation of much pigment in clusters and clumps in the spleen but not in the liver. Quincke [5] transfused into dogs blood of the dog in very large amounts. The hemoglobin content of the blood rose and was greatest on the second day, and after from two to four weeks, had returned to the normal level. After three weeks iron-containing granules, which are scant in normal animals, appeared in the cells of the red pulp of the spleen, and after four weeks some of them were found in the parenchymatous cells and in endothelial cells (Kupffer cells) of the liver. The orange-yellow pigment remained in the pulp cells for many months. Boycott and Douglas [6a] transfused 50 cc. of citrated whole blood from one rabbit into another and found that the total oxygen capacity of the blood of the rabbit that received the transfused blood was increased for a time but returned to normal after about fifteen days. In a second series of experiments, Boycott and Douglas [6b] observed an increase in the size of the spleen and leukocytosis after transfusion. Hemoglobinemia and hemoglobinuria could not be found in the animals that received the transfused blood.

Rous [7] injected into the rabbit 10 cc. of whole blood of the rabbit daily for several weeks. In microscopic sections of the spleen, phagocytosis was increased only slightly, but fragments of erythrocytes (schizocytes) accumulated in the spleen and were not washed out by perfusion. On the other hand, in the spleens of some animals that received the transfused blood extracellular masses of brown iron-containing pigment were found. Rous and Oliver [8] administered to rabbits daily transfusions of from 10 to 15 cc. of citrated whole blood of rabbits. After a few weeks of this plethora the spleen was enlarged and crowded with cellular débris and phagocytes containing red blood cells. There was siderosis of the bone marrow and of the lymph nodes. After several months the liver and kidney contained in their cells brownish yellow pigment giving the reactions of iron, and the spleen had diminished in size.

Rous and Robertson [9] studied the fate of erythrocytes in the spleens of normal animals. The spleen and other organs were perfused. The spleens of guinea pigs were always rich in phagocytes, and many phagocytes came away with the perfusion fluid. In the spleens of rabbits, man and monkey, a few phagocytes were found, and a few came away. In the spleen of a dog there were numerous phagocytes, but none appeared in the washings. In cats there was complete lack of phagocytes in the spleen, but fragments of erythrocytes could be found in the perfusion fluid

3. Cary, W. E.: J. Infect. Dis. **17**:432, 1915.

4. Hunter, W.: Lancet **2**:1209, 1258, 1315 and 1371, 1892.

5. Quincke, H.: (*a*) Deutsches Arch. f. klin. Med. **25**:567, 1880; (*b*) **27**:193, 1880.

6. Boycott, A. E., and Douglas, C. G.: (*a*) J. Path. & Bact. **13**:414, 1909; (*b*) **14**:294, 1910.

7. Rous, P.: J. Exper. Med. **25**:665, 1917.

8. Rous, P., and Oliver, F.: J. Exper. Med. **28**:629, 1918.

9. Rous, P., and Robertson, O.: J. Exper. Med. **25**:651, 1917.

from the organ and in the blood from the normal heart. Later, fragments of erythrocytes were found in the blood of every animal that was studied, and it was shown that they accumulated in the spleen.

Paton and Goodall [10] found that removal of the spleen was not followed by a rise in the number of erythrocytes or in that of leukocytes. Erythrocytes of defibrinated blood, injected into the circulation of animals of the same species, disappeared at the same rate in the animal with a spleen and in that with the spleen removed. These observations suggested that the spleen did not actively destroy blood corpuscles. They obtained evidence, they believed, that the spleen retained iron from the hemoglobin to be used in the regeneration of the blood cells. This conclusion was drawn from the observation that anemia induced by feeding rabbits a diet poor in iron and protein (rice) was more advanced in splenectomized animals. Bain [11] passed blood of a dog through the surviving spleen of the same animal for about two hours. There were fewer erythrocytes in the blood that left the spleen than in that which entered it, and there was a considerable diminution of the number of polymorphonuclear leukocytes, but the number of lymphocytes was unaffected.

Steudemann [12] produced severe congestion of the spleen by ligating the splenic vein and found that phagocytes containing red and white blood cells appeared in large numbers in the pulp. Free polymorphonuclear leukocytes in large number underwent disintegration in the spleen.

Naegeli [13] found that destruction of leukocytes took place in lymph nodes, in the spleen, in the liver and in the bone marrow. In these organs he found leukocytes and remnants of leukocytes within macrophages, often in the same cell with remains of red blood cells. Seyderhelm and Oestreich [14] stated that the occurrence of fragments of granulocytes in phagocytes of the spleen, bone marrow and lymph nodes was so infrequent that it was not significant. Noting that dying granulocytes took up congo red, they allowed pus from an empyema to stand with congo red and injected suspensions of the washed leukocytes thus obtained into the blood streams of rabbits and dogs. When the animals were killed immediately after the injection, the capillaries of the lungs contained stained leukocytes in great quantity. Such leukocytes were moderately abundant in groups in the pulp of the spleen, and some appeared in the kidney, in the bone marrow, in the liver and in the muscles. When the stained granulocytes were injected into the portal vein, they were retained in the liver, but a few hours later had disappeared. The authors reached the conclusion that dead cellular material is removed from the blood chiefly by the liver.

## METHODS

Blood was obtained from the etherized rabbit by intracardial puncture with aseptic precautions, coagulation being prevented by heparin or citrate solution. The blood was centrifugated, and the corpuscles were washed three times with salt solution. The suspension of blood cells was brought to the original volume of the blood by the addition of physiologic solution of sodium chloride.

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10. Paton, D., and Goodall, A.: J. Physiol. **29**:411, 1903.
11. Bain, W.: J. Physiol. **29**:352, 1903.
12. Steudemann, K.: Folia haemat. **18**:140, 1914.
13. Naegeli, O.: Blutkrankheiten und Blutdiagnostik, ed. 5, Berlin, Julius Springer, 1931, p. 214.
14. Seyderhelm, W., and Oestreich, C.: Ztschr. f. d. ges. exper. Med. **56**:503, 1927.

The suspension of blood corpuscles (20 cc.) was injected into the lateral ear vein. The weight of the rabbit used was in most instances about 1,500 Gm. Only in three instances, in early experiments, did the rabbit weigh 2,000 Gm. The animals were killed with ether thirty minutes, two and a half, six, eight, sixteen, eighteen, twenty-four, twenty-nine, forty-eight and ninety-six hours after the injection. The urine of these animals was tested for hemoglobinuria, and none was found. Five normal rabbits were used as controls. The spleens of all the animals were perfused with a solution containing 0.9 per cent sodium chloride, passed through the organ at a pressure of between 100 and 120 cm. of water. With the spleen still in the body, the aorta was ligated above the renal vessels, and branches of the celiac artery, with the exception of the splenic, were ligated. The rami gastrici passing to the stomach from the splenic arteries and veins were doubly ligated and severed. A glass cannula was introduced into the aorta below the diaphragm, and fluid was allowed to flow into the aorta, the splenic artery being its only outlet. No air bubbles were permitted to enter the vessels. The fluid that flowed from the splenic vein was collected for examination. The spleen was perfused until it lost its bluish red color and became pale. The perfusion fluid usually became clear after about five minutes and then a solution containing 1 part of solution of formaldehyde U. S. P. and 9 parts of 3 per cent potassium bichromate was injected during ten minutes through the cannula used for perfusion.

The perfusion fluid was centrifugated, and smears were made of the precipitated blood cells. Smears were occasionally prepared from the washed splenic tissue before it was fixed. All these smears were stained by Wright's method. Splenic tissue was fixed in solution of formaldehyde U. S. P. diluted 10 per cent in Zenker's solution and in the formaldehyde bichromate solution. The sections were stained with hematoxylin, eosin and azure, hematoxylin and eosin, or eosin and methylene blue. Sections were cut 2 microns in thickness, thinness being essential.

Perfusion of the spleen washed away not only the red corpuscles of the sinuses but those of the splenic cords as well. The perfusion fluid evidently passes so freely into the splenic pulp outside of the sinuses that it removes the erythrocytes. Observations that will be described show that few of the phagocytes either of the sinuses or of the splenic cords are dislodged by perfusion. By removing the erythrocytes perfusion greatly facilitates the recognition of phagocytes.

## PHAGOCYTES OF THE SPLEEN

The interrelation and functional significance of the cellular elements of the spleen are not clearly definable with present knowledge of the subject. Since the phagocytes of the normal spleen and of the spleen of an animal that has received blood corpuscles are the same, it is desirable to describe these cells.

*Phagocytes of the Splenic Cords.*—Mononuclear cells of small size situated in the cords of the splenic pulp often contain single engulfed erythrocytes. They constitute a large proportion of the phagocytes of the normal spleen, and it seems probable that many red corpuscles are ingested and destroyed singly. The phagocyte is often so small that its cytoplasm forms a narrow and inconspicuous zone about the nucleus and the ingested corpuscle. The nucleus of this cell stains more deeply and

homogeneously than that of the larger phagocyte. The engulfed cell is pressed against the nucleus and often lies partially within an indentation of the nuclear membrane.

Within the splenic pulp are cells of very large size and varied forms, containing blood elements. These phagocytes are found throughout the splenic cords and in the malpighian follicles, although they are much less numerous in the latter. They vary widely in shape, the outline being oval or polygonal or greatly elongated. Characteristic of these cells are their cytoplasmatic processes, which may be single or multiple and sometimes give the cell a stellate outline. Some of the processes are much elongated and divide into delicate branches. One may assume that these processes anchor the cell in some degree and that the free mobile phagocytes are those with short ameboid projections. The relation of these cells of the pulp to the reticular framework of the tissue has not been satisfactorily defined, but it is evident that both the cell body and its processes are in intimate contact with the reticular fibers.

The large vesicular, palely stained nucleus of the large phagocyte differs conspicuously from the homogeneous, deeply stained nucleus of the small phagocytic cell that contains a single erythrocyte. It is usually in the center of the cell, surrounded by inclusions, but may be eccentrically placed and occasionally is indented and distorted by the inclusions. Within the phagocytes are red blood corpuscles, pale yellow pigment derived from them, granulocytes and products of the disintegration of the latter.

*Phagocytes of the Sinuses.*—The cells that have part in forming the lining of the venous blood sinuses often contain inclusions identical with those in the phagocytes of the splenic cords. The lining cells of the sinus, it is well known, are elongated longitudinally, and their nuclei, which are drawn out in the same direction, are usually flat but sometimes bulge slightly into the lumen. Cells attached to the sinus wall that contain ingested blood cells or particles derived from them project conspicuously into the lumen. Their shape varies much, and sometimes one of them may be attached by a broad base, whereas another maintains its connection with the lining only by a narrow cytoplasmatic process. Finally these phagocytes become free in the lumen of the sinus and assume a rounded form. Erythrocytes or granulocytes are often seen attached to the surface of lining cells, but it may be difficult to decide whether they are still outside or already within the cytoplasm of the cells.

Within the lumens of the sinuses are unusually large free phagocytes, the outlines of which are rounded but slightly irregular. In some instances the free phagocyte has a single short process, and in others, several processes, the latter giving the cell an undulating contour. Occasionally a process is elongated and finger-like. The nucleus stains deeply

and has a definite nuclear membrane; it varies much in shape, being round or irregular, oval or elongated and sausage shaped; it may be large or small in relation to the cell body and is usually near the center of the cell, with the inclusions about it. These cells ingest red blood corpuscles and granulocytes and contain a variety of inclusions derived from one or another of these blood cells.

The phagocytic cells of the sinusal lining, attached or free, are not so abundant as those of the pulp cords (table 1). In a few sections some of these cells, loaded with cellular inclusions, have been seen apparently migrating through the wall of the sinus, because part of the cell is within and part outside of the sinus.

TABLE 1.—*Counts of Phagocytes in the Perfused Spleens of Normal Rabbits and of Rabbits That Received Injections of Blood Corpuscles*

| | Phagocytes in Pulp Cords | Phagocytes Attached to Sinus Walls | Phagocytes Free in Sinuses | Total |
|---|---|---|---|---|
| Controls: | | | | |
| 1 | 30 | 10 | 9 | 49 |
| 2 | 20 | 7 | 10 | 37 |
| 3 | 26 | 5 | 6 | 37 |
| 4 | 36 | 6 | 8 | 50 |
| Average of controls | 28.0 | 7.0 | 8.1 | 43.2 |
| Animals given blood corpuscles intravenously and killed at stated intervals: | | | | |
| 1 (30 min.) | 42 | 6 | 19 | 67 |
| 2 (2½ hr.) | 57 | 4 | 7 | 68 |
| 3 (6 hr.) | 36 | 4 | 10 | 50 |
| 4 (8 hr.) | 40 | 4 | 6 | 50 |
| 5 (16 hr.) | 90 | 16 | 14 | 120 |
| 6 (16 hr.) | 119 | 17 | 26 | 162 |
| 7 (18 hr.) | 115 | 16 | 19 | 150 |
| 8 (24 hr.) | 38 | 9 | 5 | 52 |
| 9 (29 hr.) | 69 | 4 | 2 | 75 |
| 10 (48 hr.) | 37 | 9 | 10 | 56 |
| 11 (96 hr.) | 39 | 6 | 3 | 48 |

## PHAGOCYTOSIS IN THE NORMAL SPLEEN

In sections of the spleens of the control animals, phagocytic cells containing red blood corpuscles, pigment or polymorphonuclear leukocytes are usually found within the splenic cords and less frequently either attached to the lining of the sinuses or free within their lumens. A single red blood corpuscle is often found in a monocyte of small size. A few phagocytes of larger size, each containing several red blood corpuscles (three or four), are found in the splenic pulp of normal animals. Pale yellow pigment in coarse granules or fine particles, which does not give an iron reaction when tested by the potassium ferrocyanide method of Perls and the ammonium sulfide and potassium ferricyanide method of Turnbull, is doubtless derived from the red blood corpuscles. Extracellular clumps of similar pigment are not infrequently seen in the splenic cords.

Pigment containing iron has been found in the spleen of one normal animal. It is noteworthy that Hunter [4] observed little iron-containing pigment in the spleens of the healthy young mammals, including rabbits, that he studied, but it was present after administration of blood poisons, namely, toluylenediamine and pyrogallol, and after transfusion of blood. Quincke [5b] found minute granules of iron-containing pigment in the spleens of healthy dogs. Rous [7] stated that small extracellular aggregations of amorphous brown pigment were abundant in spleens of rabbits into which blood had been transfused daily for a period of a fortnight. Addison [2] found in the spleens of all normal rabbits cells containing hemosiderin inclusions. Boycott and Douglas [6b] observed pigment giving the iron reaction in the spleens of all full grown rabbits (3,000 Gm.), but in small animals this pigment was absent.

Entire granulocytes (polymorphonuclear leukocytes) are frequently recognizable in large mononuclear cells of the pulp. The cytoplasm of some of these phagocytes is not filled by the leukocytes they contain, even though a considerable number are ingested. Phagocytes containing leukocytes are usually much larger than those containing red blood corpuscles. Various phases in the intracellular digestion of leukocytes are found in the normal spleen, but are much more readily studied in the spleen of the animal that has received blood corpuscles intravenously. In one stage of the process globules of basophilic material, presumably derived from the nuclei of the granulocytes, are seen within phagocytes. The accumulation of granulocytes in groups within the splenic cords will be discussed later. Myelocytes are readily found in the spleens of normal animals.

### PHAGOCYTOSIS AFTER INJECTION OF BLOOD CORPUSCLES INTO THE CIRCULATING BLOOD

The spleens of the rabbits that had received injections of blood corpuscles were slightly enlarged. They were measured, but it was not possible to weigh them properly because they had been perfused before they were removed from the animals. The average size of three normal spleens was 4.3 by 0.6 by 0.2 cm., whereas that of eight spleens after the injection of blood corpuscles was 5.3 by 0.94 by 0.3 cm.

These spleens from rabbits that had received injections of blood corpuscles were darker red than normal and appeared to be congested. Abundance of blood in the splenic cords and distention of the splenic sinuses were evident microscopically. In the perfused spleens red blood corpuscles remained in abundance in the marginal zones around the malpighian follicles, although they usually were almost completely removed from the pulp elsewhere.

Counts were made to determine the relative numbers of phagocytes of different kinds and with different contents in a measured area in

sections of approximately the same thickness. They were made in an area exposed by moving a field of the oil immersion lens (homogeneous immersion 1.8 mm., aperture 1.30 and ocular no. 2) through a distance of 1 cm. In table 1 are counts showing the relative numbers of phagocytes in the spleens of normal animals and of animals at different intervals after the injection of corpuscles. Separate enumerations were made of the phagocytes in the pulp cords, those attached to the walls of the sinuses and those free within the lumens of the sinuses. Table 2 shows the number of phagocytes containing red blood corpuscles, pigment, ingested granulocytes and nuclear fragments in the spleens of the controls and of the animals given injections of blood corpuscles.

TABLE 2.—*Counts of Phagocytes Containing Erythrocytes, Granulocytes and Products Derived from Them in Spleens of Normal Rabbits and of Rabbits That Received Injections of Blood Corpuscles*

| | Phagocytes Containing | | | |
|---|---|---|---|---|
| | Erythrocytes | Blood Pigment | Granulocytes | Nuclear Fragments |
| Controls: | | | | |
| 1 | 14 | 4 | 15 | 16 |
| 2 | 7 | 23 | 7 | 4 |
| 3 | 12 | 23 | 8 | 8 |
| 4 | 10 | 11 | 7 | 8 |
| Average of controls | 10.75 | 15.2 | 9.5 | 9.0 |
| Animals given blood corpuscles intravenously and killed at stated intervals: | | | | |
| 1 (30 min.) | 5 | 9 | 25 | 30 |
| 2 (2½ hr.) | 16 | 31 | 9 | 16 |
| 3 (6 hr.) | 13 | 10 | 13 | 16 |
| 4 (8 hr.) | 12 | 6 | 18 | 16 |
| 5 (16 hr.) | 35 | 33 | 44 | 45 |
| 6 (16 hr.) | 89 | 34 | 28 | 6 |
| 7 (18 hr.) | 32 | 54 | 32 | 30 |
| 8 (24 hr.) | 11 | 22 | 11 | 10 |
| 9 (29 hr.) | 7 | 20 | 18 | 27 |
| 10 (48 hr.) | 16 | 6 | 19 | 17 |
| 11 (96 hr.) | 10 | 12 | 17 | 11 |

In animals killed thirty minutes after the injection of blood corpuscles phagocytosis in the spleen was already more active than in the normal animals. At this time there was an increase in the phagocytosis of granulocytes, and after two and a half hours there was some increase in that of erythrocytes. Phagocytosis of both white and red cells maintained average rates slightly above the control rates during eight hours after the injection, but sixteen and eighteen hours after the injection the activity of the process increased notably and then diminished so that after twenty-four hours it was approximately at, or slightly above, the normal level. The number of phagocytes containing disintegration products of erythrocytes, represented by yellow pigment, exhibited fluctuations at different intervals of time after the injection of corpuscles, but these were perhaps not significant, and phagocytes containing pigment were increased notably only in the animal killed after eighteen hours. Disin-

tegration products of granulocytes, represented by nuclear fragments within phagocytes, were increased in the animal killed after thirty minutes, were most abundant after sixteen hours and maintained for forty-eight hours a level above that of the controls.

It is noteworthy that phagocytosis of both erythrocytes and granulocytes and evidence of intracellular digestion were most conspicuous from sixteen to eighteen hours after the introduction of blood corpuscles into the blood stream. This increased phagocytic activity affected chiefly the phagocytes of the pulp cords, though the phagocytes of the sinuses as well attained maximal numbers sixteen and eighteen hours after the injection of corpuscles.

TABLE 3.—*Minimal, Maximal and Average Diameters of Phagocytes of the Spleens of Normal Rabbits and of Rabbits That Received Injections of Blood Corpuscles*

| | Minimum, Microns | Maximum, Microns | Average, Microns |
|---|---|---|---|
| Controls: | | | |
| 1 | 8.6 by 5.7 | 14.3 by 14.3 | 13.2 by 9.2 |
| 2 | 9.6 by 8.0 | 24.1 by 11.2 | 14.4 by 9.6 |
| 3 | 9.6 by 8.0 | 22.4 by 14.5 | 14.5 by 9.6 |
| 4 | 9.6 by 8.0 | 19.2 by 11.2 | 12.4 by 8.9 |
| Average of controls | 9.3 by 7.5 | 20.0 by 12.5 | 13.6 by 9.3 |
| Animals given blood corpuscles intravenously and killed at stated intervals: | | | |
| 1 (30 min.) | 10.0 by 5.6 | 25.7 by 11.4 | 15.4 by 10.2 |
| 2 (2½ hr.) | 9.6 by 9.6 | 22.4 by 16.1 | 14.9 by 10.3 |
| 3 (6 hr.) | 8.0 by 6.4 | 22.4 by 17.7 | 13.4 by 10.4 |
| 4 (8 hr.) | 12.8 by 9.6 | 52.2 by 11.3 | 17.0 by 11.2 |
| 5 (16 hr.) | 9.6 by 8.0 | 22.5 by 14.5 | 17.9 by 11.2 |
| 6 (16 hr.) | 12.8 by 9.7 | 27.4 by 16.1 | 17.6 by 12.5 |
| 7 (18 hr.) | 11.3 by 9.7 | 28.6 by 14.5 | 16.2 by 12.0 |
| 8 (24 hr.) | 11.2 by 6.4 | 22.5 by 19.3 | 14.6 by 9.5 |
| 9 (29 hr.) | 9.6 by 9.6 | 19.3 by 16.1 | 13.6 by 9.4 |
| 10 (48 hr.) | 8.0 by 4.8 | 17.7 by 14.5 | 12.8 by 9.6 |
| 11 (96 hr.) | 9.6 by 9.6 | 16.1 by 11.2 | 13.6 by 8.8 |

In order to determine whether the phagocytes increased in size as the result of greater phagocytic activity following the injection of blood corpuscles, 20 phagocytes were measured in sections from normal rabbits and rabbits given injections of blood corpuscles. In table 3 are given the minimal, the maximal and the average diameters thus obtained. It is evident that large phagocytes appeared eight hours after the introduction of corpuscles into the circulation and persisted up to eighteen hours, but that after twenty-four hours the phagocytes of the spleen had returned to approximately normal dimensions.

### PHAGOCYTES IN PERFUSION FLUID

Study of the perfusion fluid that was recovered from the splenic vein showed that few phagocytes came away in the fluid that had washed the spleens. The cells that were flushed out were in almost every instance

large rounded phagocytes, identical with the large free phagocytes of the venous sinuses. In some specimens phagocytes were found only after prolonged search or not at all. They contained red blood cells, granulocytes and vacuoles in their cytoplasm. In the perfusion fluid were many lymphocytes, small, medium and large, and some lymphoblasts.

An attempt was made to determine whether the numbers of phagocytes in these spleens were diminished by perfusion. In part of the experiments the branches of the splenic artery to the caudal half of the spleen were ligated before perfusion so that only the other half of the organ was perfused. Microscopic sections from the two parts were compared. Table 4 gives the results of counts of phagocytes in the unwashed splenic tissue. These counts are from animals that were killed at the same intervals of time as some of those in table 1. It is more

TABLE 4.—*Counts of Phagocytes in Splenic Tissue with No Perfusion from Normal Rabbits and from Rabbits That Received Injections of Blood Corpuscles*

| | Phagocytes of Pulp Cords | Phagocytes of Sinus Wall | Phagocytes Free in Sinuses | Total Phagocytes |
|---|---|---|---|---|
| Controls: | | | | |
| 1 | 24 | 3 | 10 | 37 |
| 2 | 40 | 7 | 5 | 52 |
| Average of controls | 32.0 | 5.0 | 7.5 | 44.5 |
| Animals given blood corpuscles intravenously and killed at stated intervals: | | | | |
| 1 (30 min.) | 33 | 5 | 24 | 62 |
| 2 (2½ hr.) | 59 | 6 | 9 | 74 |
| 3 (6 hr.) | 40 | 6 | 14 | 60 |
| 4 (16 hr.) | 94 | 8 | 12 | 114 |
| 5 (16 hr.) | 81 | 12 | 25 | 120 |
| 6 (24 hr.) | 35 | 5 | 5 | 45 |
| 7 (48 hr.) | 37 | 5 | 7 | 49 |
| 8 (96 hr.) | 39 | 6 | 3 | 48 |

difficult to identify phagocytes in the unwashed tissue, and the figures obtained are not as accurate as those from perfused spleens. Comparison of tables 1 and 4 shows that no noteworthy difference in the number of phagocytes in washed and unwashed tissues has been found.

## INTRACELLULAR DESTRUCTION OF RED CORPUSCLES

Phagocytic cells containing erythrocytes were more numerous in the splenic cords than in the sinuses. Many of these cells had protoplasmatic processes, but others of very large size were irregularly rounded and evidently free in the pulp. Some phagocytes that had ingested red corpuscles contained, in addition, masses of pale yellow granular pigment, and some of these cells attained great size. Free phagocytic cells containing erythrocytes were fewer than those that were still attached to the lining. Erythrocytes evidently adhered to the surface of the phagocytes before they were ingested and sometimes 1, 2 or many red cells were found in contact with a large cell free within a sinus.

Red blood corpuscles, pigment, granulocytes and products of their disintegration were sometimes found in a single phagocyte.

The ingested red cells evidently became smaller and spherical and at times colorless. In some instances they took a deep dull red stain with eosin, and sometimes outlines of individual corpuscles were lost as though they were fused. The transformation of the blood disks into amorphous or granular pigment presumably proceeded rapidly, for no transitions were found between the two. The blood pigment appeared in two forms: as sharply outlined round or irregular pale yellow globules, easily identified by their light color and shining appearance, or as very fine dustlike particles of the same color scattered throughout the cytoplasm of the cell. The two kinds of pigment were sometimes present together in the same cell.

In the experiments that have been described in this paper the light yellow pigment that was formed failed to give the reactions of iron, and no hemosiderin was demonstrable within ninety-six hours after the intravenous administration of blood corpuscles. Quincke [5b] examined the spleens of dogs at intervals of from five to two hundred days following transfusion of large quantities of blood of dogs and found no increase of iron-containing pigment in the spleen until two or three weeks later. These experiments did not demonstrate whether the red cells which underwent phagocytosis were already damaged and moribund. It could not be determined whether the engulfed cells were the rabbit's own cells or the injected blood corpuscles, but in view of their rapid increase after injection it is probable that they were in large part the injected corpuscles. Rous,[15] reviewing the subject, found no characters by which the oldest erythrocyte could be recognized.

### GROUPS OF GRANULOCYTES IN SPLENIC CORDS

When sections of the spleens were examined by low power, small spots brightly stained with eosin were seen both in controls and in experimental animals, but much larger in the latter. Examined by high power they were found to be groups of granulocytes in the splenic cords of the red pulp, those in the controls being comprised of from approximately 3 to 7 granulocytes and those in animals that had received blood corpuscles, of as many as from 10 to 20 or more granulocytes. Many of these leukocytes were obviously in a process of disintegration. Their nuclei were fragmented and deeply stained. The lobes of the polymorphous nucleus in some instances separated from one another, and the remnants assumed a globular form. The specific granules of the cell lost their regular form, and coarse granules deeply stained with eosin appeared. Finally the granules in some instances were scattered and the

15. Rous, P.: Physiol Rev. **3**:75, 1923.

cell outline disappeared completely. Products of disintegration of leukocytes, recognized as scattered specific granules or as nuclear particles, were sometimes found in considerable abundance between the cells and surrounding the group of them.

Phagocytes of the splenic cords with swollen vesicular nuclei were usually seen within and about these groups and apparently ingested some of the fragments of the cells and even whole granulocytes. It was often difficult to determine what was the relation of the granulocytes of these groups to phagocytes, because it was not improbable that in many instances the granulocytes were adherent to the surfaces of phagocytes, and in other instances it was possible that some of the granulocytes of the groups were actually within phagocytes whose vacuolated cell bodies were not clearly outlined.

### INTRACELLULAR DISINTEGRATION OF GRANULOCYTES

Granulocytes, like erythrocytes, were seen in immediate contact with large cells, and it is probable that they stuck to the surface before they were ingested. Within the phagocytic cells, the nuclei of the granulocytes sometimes increased in size and stained deeply and homogeneously with hematoxylin. In some instances lobes of a nucleus seemed to separate from one another, the nuclear bridges being lost. Sometimes a nucleus broke into numerous fragments of different sizes, scattered as pyknotic particles either within the still intact granulocyte or later in the cytoplasm of the phagocyte. In some instances the outline of a granulocyte disappeared in an early phase of disintegration; in others it persisted until neither nucleus nor granules were recognizable. Sometimes granulocytes, in various stages of disintegration, lay within clearly definable vacuoles.

In some instances the granules of intracellular granulocytes lost their regular distribution and diminished in number. They varied in size, some large globules being seen. Finally they might form a small compact mass of granules. The acidophilic granules in most instances remained intact longer than the nucleus of the cell, and they were sometimes found free in the cytoplasm of the phagocyte. In the latest stages of intracellular disintegration the phagocyte might contain a few globules lightly stained with eosin and some nuclear fragments scattered throughout the cytoplasm of the phagocyte.

A conspicuous feature of the intracellular digestion of granulocytes was the formation of sharply defined globular particles of basophilic material stained deeply on the periphery and clear in the center. They were evidently derived from nuclear material, but how they were formed from the nucleus is not clear. They sometimes lost their basophilic stain and took up eosin, but all transitions from one to the other were occa-

sionally found in the same field. The particles had the same size or were slightly larger than the lobes of the nucleus of the granulocyte. These homogeneous globular particles might be found in the granulocyte, often close to its periphery or free within the cytoplasm of the phagocyte.

## SUMMARY

Perfusion of the spleen washes red blood corpuscles and lymphocytes from both the splenic cords and the sinuses but in large part leaves mononuclear phagocytes and granulocytes undisturbed.

In the spleen of normal rabbits there is active phagocytosis and intracellular digestion of both erythrocytes and granulocytes by phagocytes of the splenic cords and, in smaller number, of the sinuses.

When blood corpuscles of the rabbit are introduced into the blood stream of the rabbit phagocytosis of erythrocytes and granulocytes is greatly increased and reaches a maximum at between sixteen and eighteen hours after the injection.

Within the phagocyte erythrocytes stain deeply with eosin and apparently fuse to form globular masses, but these changes are transient and are promptly followed by formation of pale yellow pigment, which has failed to give the reactions of iron.

Small groups of granulocytes occur in the splenic cords of the normal rabbit, and after injection of homologous blood corpuscles groups of granulocytes, in part undergoing disintegration, are for a time more numerous and larger than those in the normal spleen.

Intracellular digestion of granulocytes is characterized by clumping and solution of specific granules, by fragmentation, pyknosis and solution of nuclear substance, and by formation from nuclear material of globules that tend to lose their basophilic character and stain with acid dyes.

# Case Reports

## HEMOCHROMATOSIS WITH PRIMARY CARCINOMA OF THE LIVER

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In his recent monograph, Sheldon [1] reviewed the 345 cases of hemochromatosis which have been reported and accepted 311 as authentic. To this number he added the 52 cases reported by Stewart,[2] which were found in a series of 38,095 autopsies in five British hospitals, making a total of 363 accepted cases. Altogether there were 26 cases of associated primary carcinoma of the liver (7.1 per cent). Stewart found that in 124 cases of cirrhosis, after eliminating those in which the slighter grades were present, the incidence of carcinoma of the liver was 7.3 per cent. As the percentages of incidence in the two conditions are almost identical, these authors concluded that the cirrhosis, rather than the pigmentation, was responsible for the malignant changes of the liver found in 26 cases of hemochromatosis.

Sheldon, in summarizing 20 cases of primary carcinoma of the liver associated with hemochromatosis, showed that in most instances the tumor was a hepatoma, that usually the hepatoma invaded the portal veins and that in 5 cases it metastasized. Metastasis was most often to the regional lymph glands; occasionally, to the pancreas, peritoneum, pleura, bronchial glands and lungs. The most significant histologic feature was the absence of pigment from the malignant cells. These contained hemosiderin in only 2 cases.

A review of the literature of hemochromatosis since Sheldon wrote his monograph reveals 23 acceptable cases; in 20 of these there was an autopsy. Three presented a typical clinical picture, and biopsy of the skin demonstrated much iron pigment in the corium. Only 1 case

From the National Institute of Health, Washington, D. C., and the United States Marine Hospital, Baltimore.

1. Sheldon, J. H.: Haemochromatosis, New York, Oxford University Press, 1935.

2. Stewart, M. J.: Lancet **2**:565, 1931.

occurred in a female. It is of interest that in a case of tularemia reported by Beck and Merkel [3] hemochromatosis was shown post mortem. As the term "hemochromatosis" did not appear in the title the case had not been indexed as one of hemochromatosis.

In 2 of these 23 additional cases a tumor of the liver was recorded:

Meldahl [4] reported a case of hemochromatosis in a 64 year old man. The liver was cirrhotic, weighed 2,235 Gm., contained much hemosiderin and presented a walnut-sized soft white tumor which was diagnosed as simple adenoma. The cells of the adenoma were pigment free. The histologic description was very brief.

*Cases of Hemochromatosis Reported Since Sheldon's Monograph*

| Author | Sex | Age | Comment |
|---|---|---|---|
| Bouchut, L., and others: J. de méd. de Lyon **16**: 611, 1935 (1) | M | 40 | Autopsy |
| (2) | M | 33 | Autopsy |
| Cabot Case 21251, New England J. Med. **212**: 1178, 1935 | M | 36 | Autopsy |
| Soper, V.: M. Rec. **142**: 130, 1935 | M | 57 | Biopsy of skin—much hemosiderin in corium |
| Labbé, M., and Petresco, M.: Ann. d'anat. path. **11**: 761, 1934 | M | 63 | Autopsy |
| Meldahl [4] (1) | M | 45 | Autopsy |
| (2) | M | 64 | Autopsy—adenoma hepatis |
| (3) | M | 55 | Autopsy |
| (4) | M | 55 | Autopsy |
| Donzelot, E.: Arch. d. mal. du cœur **29**: 1, 1936 | M | 38 | Autopsy |
| Labbé, M., and others: Presse méd. **44**: 537, 1936 | M | 42 | Autopsy |
| Cantoni, O.: Clin. med. ital. **66**: 935, 1935 | M | 35 | Autopsy |
| Thompson, K. S.: Birmingham M. Rev. **11**: 153, 1936 | M | 49 | Autopsy |
| Coates, F., and others: Ulster M. J. **5**: 172, 1936 | M | 38 | Autopsy |
| Miskall, E. W.: Ohio State M. J. **32**: 746, 1936 | M | 39 | Biopsy of skin—much hemosiderin in corium |
| Cicardo, V. H.: Prensa méd. argent. **23**: 1212, 1936 | M | 56 | Autopsy |
| Murray Lyon, R. M.: Brit. M. J. **1**: 1297, 1936 (1) | M | 27 | Autopsy |
| (2) | M | 54 | Autopsy |
| (3) | F | 34 | Autopsy |
| Orgel, M. N., and Barr, D.: Endocrinology **20**: 839, 1936 | M | 44 | Autopsy |
| Beck and Merkel [3] | M | 61 | Autopsy—coincidental tularemia |
| Creed, J. P.: J. A. M. A. **105**: 1185, 1935 | M | 65 | Biopsy—much hemosiderin in corium |
| Hausmann [5] | M | 64 | Autopsy—primary carcinoma of liver |

Hausmann [5] reported the case of a 64 year old man who presented a dark brown pigmentation of the skin, no diabetes, cirrhosis of the liver with ascites, and a very dark colored urine. The urinary pigment was thought to be melanin. At autopsy there was cirrhosis of the liver and pancreas. In addition, the liver showed yellow-gray nodules measuring up to 4 cm. in diameter, which histologically were composed of large carcinomatous cells thought to have originated from intrahepatic bile ducts. He stated that there was an enormous amount of iron in the liver cells, less in the small bile ducts and even less in the carcinomatous cells. Iron was also found in the lymph nodes and pancreas. No melanin was seen. There were no metastases.

3. Beck, H. G., and Merkel, W. C.: South. M. J. **28**:422, 1935.
4. Meldahl, K. F.: Hospitalstid. **78**:309, 1935.
5. Hausmann, M.: Helvet. med. acta **3**:695, 1936.


Fig. 1.—*A*, tumor-free liver showing cirrhosis and pigment; reduced from × 125. *B*, carcinoma in the liver; reduced from × 250.

With the addition of these cases carcinoma has been recorded 28 times in 386 cases of hemochromatosis.

We wish to report a case of hemochromatosis with malignant hepatoma of the liver in which there was moderate pigmentation of some of the malignant cells both in the liver and in the metastases in the lymph glands.

W. E., a white man aged 57, a laborer, American, was admitted to the hospital. With the exception that there had been moderate use of alcohol, the personal and family history were of no interest. The patient complained that for about five years he had been having intermittent attacks of epigastric pain, accompanied by belching and occasionally by nausea but not related to meals. Often early in the morning there was "pain in the back." During the past four months he had lost 50 pounds (22.7 Kg.) in weight and had been having polydipsia, nocturia and swelling of the ankles.

The skin of the face, neck, hands, forearms and legs showed rather marked dark brown pigmentation. There was practically no pigmentation of the skin of the trunk. Several small lipomas were found on the arms and abdomen. The heart was slightly enlarged to the left. A systolic murmur heard over the mitral area was not transmitted. The blood pressure was 114 systolic and 76 diastolic. The abdomen was smooth and moderately tender. The edge of the liver was palpable, firm and not tender. Motion in the lumbar region was slightly limited. A little tenderness was present over the sacro-iliac joints.

The urine showed sugar, a slight trace of albumin and no acetone bodies.

After repeated examinations of fresh specimens, with negative results, hemosiderin was found within the epithelial cells of one specimen (Rous test).

The Kahn reaction of the blood was negative, the sedimentation time three hours and twenty-five minutes, nonprotein nitrogen 26 mg. and sugar 281 mg. per hundred cubic centimeters, the erythrocytes 3,900,000, the hemoglobin 85 per cent, the leukocytes 4,850, and the culture negative. The Kahn reaction of the spinal fluid and the mastic test were negative.

Analysis of the gastric contents showed hypo-acidity. A liver function test with bromsulphalein resulted in 13 per cent retention of the dye in one hour.

Roentgen examinations of the teeth, chest, spine and gastro-intestinal and urinary systems revealed one periapical dental abscess, some dilatation of the ascending portion of the aorta and slight chronic arthritis.

In a biopsy of the skin a very little hemosiderin was found about the sweat glands and capillaries of the corium.

On a daily diet of 1,700 calories and 45 units of insulin the glycosuria disappeared and the blood sugar returned to normal. The pain in the back and abdomen increased in severity, and sometimes at night an opiate was required. The liver became nodular and gradually enlarged until the lower border reached the level of the anterior superior spine of the ileum. Weakness and loss of weight were progressive, and death occurred approximately three months after hospitalization.

*Autopsy.*—The skin of the face and arms had a yellowish brown tinge. The color over the rest of the body was less marked. There was brown blotchy pigmentation over both anterior tibial surfaces.

The right pleural cavity contained 3,150 cc. of dark reddish brown fluid, which had compressed the right lung. The left pleural cavity showed no fluid. The visceral pleura of both lungs was the seat of numerous flat yellowish white hard

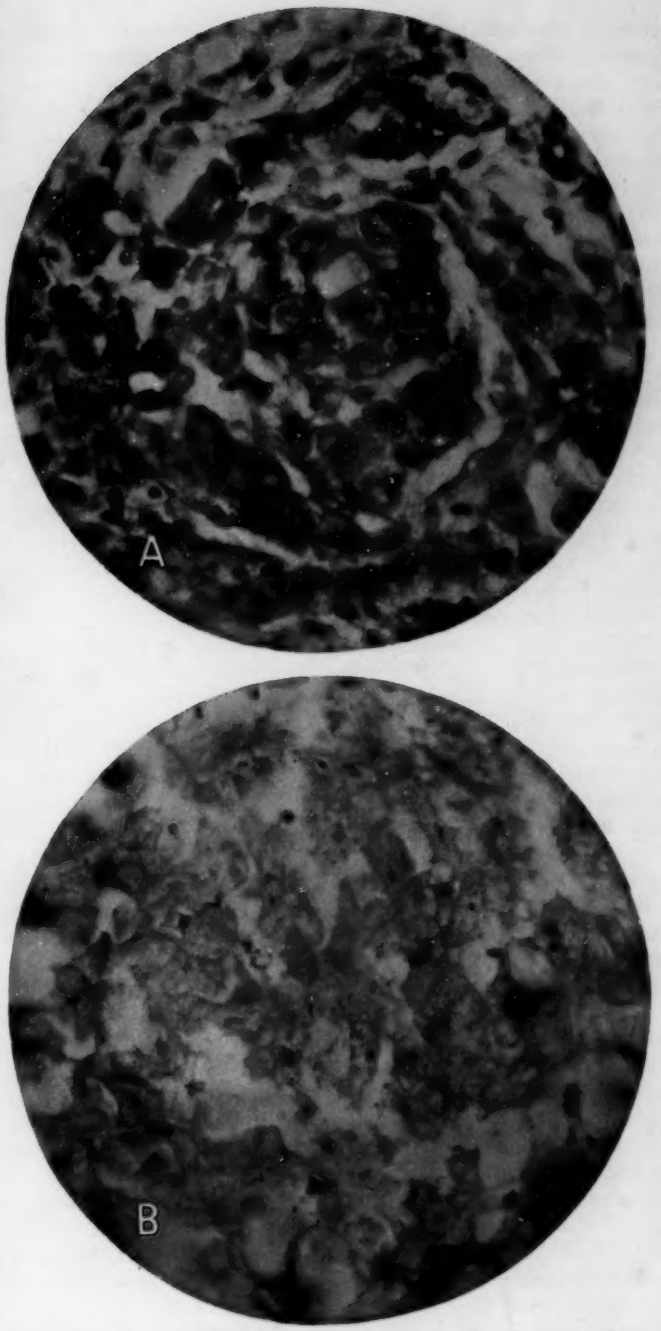


Fig. 2.—*A*, pigment-free nodule of carcinoma, surrounded by heavily pigmented liver stroma; reduced from × 475; iron stain. *B*, metastatic carcinoma in a celiac lymph node, showing fine intracellular hemosiderin granules; reduced from × 475; iron stain.

nodular lesions, which varied from 1 to 40 mm. in diameter. Similar nodules studded the tracheobronchial lymph nodes. The heart weighed 430 Gm., and the myocardium was a pale yellowish color.

The peritoneal cavity contained approximately 500 cc. of dark reddish brown fluid. The liver was greatly enlarged, extended over a handbreadth below the costal margin and weighed 4,380 Gm. The external surface was rough, irregular and dark reddish brown, and mottled with yellowish white nodules, which varied in size from 3 to 50 mm. and were located chiefly in the right lobe. The sectioned surfaces were bloody and showed many fairly large yellowish brown areas, which appeared to be surrounded by bands of connective tissue. These areas were solidly blue when tested for hemosiderin by the ferrocyanide test.

The celiac nodes were much enlarged and were infiltrated by tumor.

The pancreas weighed 200 Gm. Its shape was distorted by a series of large dark brown nodules, which averaged about 15 mm. in diameter and on section were cystic and filled with clear white mucus.

The spleen weighed 860 Gm. Three-fourths of the external surface was covered with a yellowish white exudate about 1 mm. in thickness. The pulp scraped slightly, was purple and bloody.

*Microscopic Examination.*—Wide areas of the liver were composed of small to fairly large irregular nodules of liver cords enmeshed in a hyperplastic, coarsely and finely trabeculated, densely fibrosed interlobular and intralobular stroma showing numerous irregular capillaries and ducts, many proliferating fibroblasts and moderate lymphocytic infiltration. Often amorphous masses of hemosiderin completely filled the liver cell cords of an entire nodule, or granular clusters of it occupied the central parts of their cells. The nuclei were usually pigment free. Large amounts were found in the Kupffer cells, in the epithelium of the bile ducts, and as intracellular or extracellular masses and granules in the hyperplastic periportal stroma.

In several blocks were zones in which normal bile ducts and cords appeared to be changing into irregular cords and acini with large, rounded or oval, fairly clear nuclei showing hyperchromatic stippling and relatively hyperchromatic cytoplasmic zones. In other areas the nodular growth was entirely neoplastic, replacing rather than compressing the parenchyma and invading several veins. Mitoses were fairly frequent. There were several areas of necrosis of the tumor. Only a few cells of the tumor showed significant amounts of hemosiderin, but its stroma and Kupffer cells were densely pigmented.

In the lymph nodes (celiac and tracheobronchial) metastatic cords of coherent, more or less polygonal tumor cells partly or completely replaced the parenchyma, infiltrated the capsules or invaded veins. As compared with those of the primary growth, the cells were generally larger and showed more frequent mitoses. There were areas of tumor necrosis or hemorrhage, also much fibrosis. Hemosiderin was abundant in reticulum cells, in large free phagocytes and in tumor stroma. Neoplastic cells contained but little pigment. In addition the tracheobronchial nodes showed anthracosis, and an adjacent large bronchus had small amounts of hemosiderin in and around cartilage cells and in the epithelium of the bronchial glands.

In the parietal pleura multiple sessile metastatic tumor nodules were growing on dense fibrous tissue or invading striated muscle (diaphragm). No pigment was seen in the tumor or in the muscle fibers.

The lung showed multiple small or extensive tumor nodules, measuring up to 5 mm. in thickness, growing on or invading the fibrosed pleura and adjacent alveoli. The nodules were composed of sheets or cords of cells resembling those found in

the lymph nodes. The veins, arteries and lymph spaces were often filled by tumor cells. The only deeply situated metastatic growth was within and around a large thrombosed vein.

In addition to tumor, the lung showed: patches of atelectasis, interstitial fibrosis and serosanguineous exudation; moderate numbers of bronchi filled with pus or serous exudate; relatively small amounts of hemosiderin in alveolar epithelial cells and in intra-alveolar phagocytes, and fairly numerous carbon-laden phagocytes.

The parenchyma of the pancreas was divided by copious, coarsely and finely trabeculated interlobular and intralobular fibrous stroma into many small to medium-sized nodules. The interlobular portions were permeated by numerous small ducts. Deposits of hemosiderin were dense in the acinar epithelium, less dense in the stroma and moderate in the epithelium of the larger ducts. Only an occasional group of cells resembled the islets of Langerhans.

The reticulum of the splenic pulp was thickened and slightly fibrosed. The sinuses were dilated and filled with blood or empty. In areas their endothelial cells were prominent. Relatively little hemosiderin was deposited as amorphous masses in the pulp or as granules in free phagocytes or in reticulo-endothelial cells.

The muscle fibers of the heart showed moderate hypertrophy and much hemosiderin, which varied from fine granules distributed along the myofibrils to elongated amorphous masses completely filling the fibers.

The aorta was essentially negative.

The kidneys disclosed slight fibrous thickening of the intima of arteries and arterioles and a few hyaline casts. A moderate number of convoluted tubules had intraepithelial hemosiderin granules, and the stroma of some glomerular loops was slightly pigmented.

In the adrenal hemosiderin pigmentation was marked in the cords of the zona glomerulosa and very slight in the medullary stroma.

In the appendix slight hemosiderin pigmentation was found around capillaries and small blood vessels of the submucosa. There was moderate melanosis of the mucosa.

In the urinary bladder pigmentation was moderate in the superficial mucosal stroma and slight in the interfascicular stroma.

In the brain the cuboid cells of the choroid plexus in the fourth ventricle and the anterior lobe of the pituitary (posterior lobe not seen) showed heavy hemosiderin pigmentation. There were no other lesions.

In none of the organs studied were there any significant amounts of iron-free brown pigment described by some authors as hemofuscin.

*Chemical Analysis.*—The results of quantitative chemical analysis of tumor-free liver reported by Elias Elvove, National Institute of Health, Washington, D. C., are as follows: iron, 12.08 mg. (1.21 per cent) per gram (wet); copper, 10 mg. per kilogram (wet).

Sheldon stated that wet liver normally contains from 0.05 to 0.1 per cent iron and that the average for liver affected by hemochromatosis according to the work of forty-two authors is 1.15 per cent. With reference to copper he stated that, according to reports on 71 normal livers, the average amount for wet tissue is 5.97 mg. per kilogram, while in hemochromatosis, based principally on 38 livers analyzed by Schönheimer and his school, it averages about four times the normal figure.

# PRIMARY ENDOTHELIOMA OF THE SPLEEN

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In 1923 Smith and Rusk [1] collected 104 instances of malignant tumors of the spleen from the literature and added 2 cases of their own. Several other cases have been reported [2] bringing the total to 125.

The number of primary neoplasms of the spleen has been a matter of controversy. Many, however, are apparently derived from the reticular or endothelial cells of the sinuses. The following report concerns an additional instance of such a primary endothelioma of the spleen.

## REPORT OF A CASE

A 67 year old Polish housewife was admitted to the medical service of Montefiore Hospital June 6, 1933, complaining of weakness of seven months' duration. During the past two or three years, she had noted a burning sensation of the hands and feet associated with swelling and occurring every two or three days. These symptoms were aggravated by exertion. Seven months prior to admission, she began to suffer from progressive weakness and increasing fatigability, which rapidly became worse until four months before admission, when she became bed-ridden. During the last six months, she had lost 60 pounds (27.2 Kg.) in weight. Constipation had recently become a prominent symptom, requiring the use of cathartics and frequent enemas. During the past two months, the family had observed a lemon colored pallor and some loss of memory. Her appetite failed considerably. About four months prior to admission, she was treated by a physician with injections of arsenic and iron, but no liver extract or other medication was given.

In her past history there is nothing of significance. One of her brothers died of cancer of the liver at the age of 70, and a son, of "cancer in the thigh" at the age of 34.

*Physical Examination.*—When she was admitted to the hospital, she was observed to be a cachectic and dehydrated, senile, edentulous white woman, apparently very weak and unable to cooperate. There was coarse tremor of the hands. The skin was pale. The left pupil was irregular, but both pupils reacted sluggishly to light. The surface of the tongue was smooth. Some dulness to percussion

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From the Laboratory Division of Montefiore Hospital.

1. Smith, C. E., and Rusk, C. T.: Arch. Surg. **7**:371, 1923.

2. Howard, T.: J. Lab. & Clin. Med. **14**:1157, 1929. Frank, L.: Am. J. M. Sc. **183**:77, 1932. McNee, J. W.: J. Path. & Bact. **39**:83, 1934. Stevenin, H., and others: Presse méd. **43**:382, 1935. Langerstrass, K. H., and Neumann, M.: Arch. Path. **20**:752, 1935. Paine, C. G.: J. Path. & Bact. **34**:139, 1931. Caldwell, G. T.: South. M. J. **26**:120, 1933. Krumbhaar, E. B.: Ann. Clin. Med. **5**:833, 1927. Werwath, K.: Zentralbl. f. Chir. **62**:805, 1935. Wright, J. H., and Stevenson, E. M. K.: Glasgow M. J. **114**:1, 1930.

and decrease of the breath sounds were observed at the base of the lower lobe of the left lung posteriorly. The heart was enlarged to the left. The rhythm was regular, and there was a systolic murmur over the entire precordium, loudest at the apex, but not transmitted to the axilla. The blood pressure was 104 systolic and 84 diastolic. The spleen was barely palpable, but the edge was firm. The liver was felt from 1 to 2 fingerwidths below the costal margin. An anal fistula with a purulent discharge was present. The knee jerks were sluggish. No neurologic tests requiring cooperation could be made.

There was severe secondary anemia. The hemoglobin was 35 per cent; the red blood cells, 2,700,000. No anisocytosis or poikilocytosis and no normoblasts or other cells of abnormal form were present. The leukocyte count was 18,200; the differential count showed polymorphonuclear leukocytes 56 per cent, lymphocytes 39 per cent, mononuclear cells 4 per cent and basophils 1 per cent. The serum albumin was 3.2 per cent; the serum globulin, 3.1 per cent; the total proteins, 6.3 Gm. The icteric index was 11 units; the blood sugar, urea nitrogen and blood cholesterol were within normal limits. The Wassermann reaction was negative. The stool gave a positive benzidine reaction. The urine was normal, and analysis of the gastric content revealed free hydrochloric acid. On roentgen examination of the chest, an effusion was observed at the base of the left lung, and the left leaf of the diaphragm was elevated.

*Course.*—One month after admission, the spleen was felt for the first time 5 cm. below the costal margin. The edge was firm. The patient took nourishment poorly, remaining dehydrated throughout her stay in the hospital. She was always uncooperative, irritable and noncommunicative.

Toward the end, she complained of pain in the right upper quadrant of the abdomen; the temperature was slightly elevated; the pulse rate varied between 90 and 100; and the respiration rate was from 18 to 20. The patient became progressively weaker and died July 10, 1936, five weeks after admission.

*Anatomic Diagnosis.*—Post mortem the following conditions were present: primary endothelioma of the spleen; peritonitis; perforation of the splenic flexure of the large intestine; bilateral pleural effusion; atelectasis and edema of both lower lobes of the lungs; anal fistula, and anemia. (Only the positive observations are reported.)

There was 250 cc. of clear straw-colored fluid in each pleural cavity. On the right and on the left the diaphragm was at the level of the fourth rib. Pleural adhesions were found on the surface of the left lung. The lungs were edematous and both lower lobes atelectatic.

The heart weighed 250 Gm. There was some atherosclerosis of the coronary vessels, but they remained patent.

In the abdomen, 300 cc. of greenish yellow thin fluid with a Bacillus coli odor was found. The small intestine was collapsed, but the cecum and the ascending and transverse portions of the colon were dilated. A gray-black necrotic area (4 cm. in diameter), with a perforation along the border of attachment of the omentum, was found at the splenic flexure. The peritoneum was dull and covered in many places with a fibrinoplastic exudate that peeled off easily, leaving a smooth surface.

In the left upper quadrant of the abdomen, there was a large mass (18 by 14 by 9 cm.), weighing 420 Gm., incorporating the spleen in its central portion, the diaphragm above, the splenic flexure of the colon in front and the tail of the pancreas medially. The stomach was displaced to the right; its wall was separate, distinct and easily segregated from the mass. The tail of the pancreas was firmly

adherent but could be dissected away without destroying its normal contour. The attached portion of the colon could also be separated without tearing through its wall. There was great difficulty in separating the diaphragm. The remainder of the tumor was seen to invade the spleen only, the superior portion of which had a normal appearance except in spots where the diaphragm was adherent. These areas were roughened and pale yellow. The mass itself was gray and firm in most places but soft near the upper pole. On its surface were many smaller firm gray nodules. The entire mass was covered by a thin layer of omentum that seemed to be an integral part of the outer surface of the tumor, somewhat like

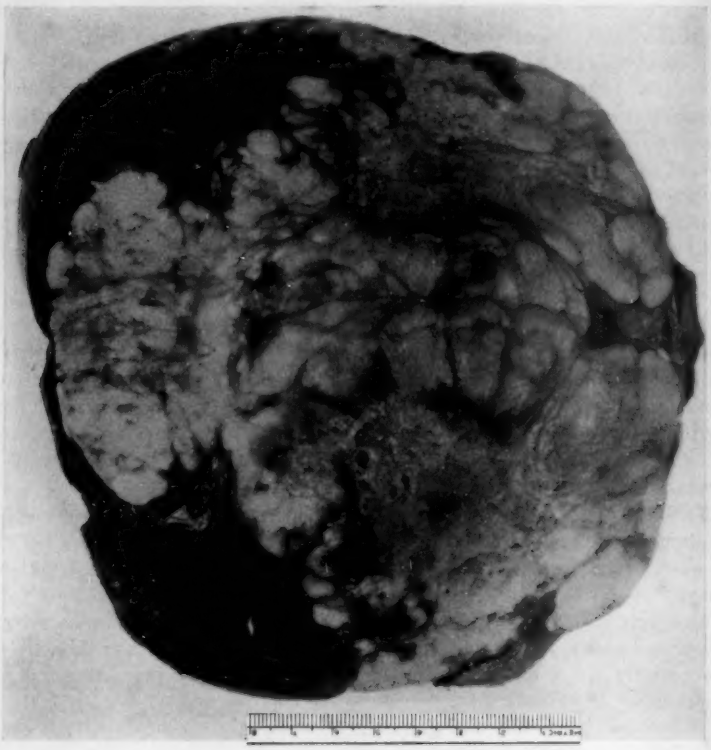

Fig. 1.—Primary endothelioma of the spleen—gross specimen.

a capsule. The cut surface revealed a large white and gray mass, firm and fibrous throughout except for a cystic degenerated area near the upper pole, from which escaped green fluid. The invading tissue was not encapsulated but became firmly interwoven with the small amount of remaining splenic pulp (about 8 mm.) at the upper pole of the mass. Section of the smaller tumors on the periphery showed gray, firm, somewhat elastic tissue, well delineated on the outer edge, but an actual part of the larger mass beneath.

There were several enlarged lymph nodes in this region retroperitoneally and along the splenic vessels. No other masses of the same or similar nature were found elsewhere in the body.

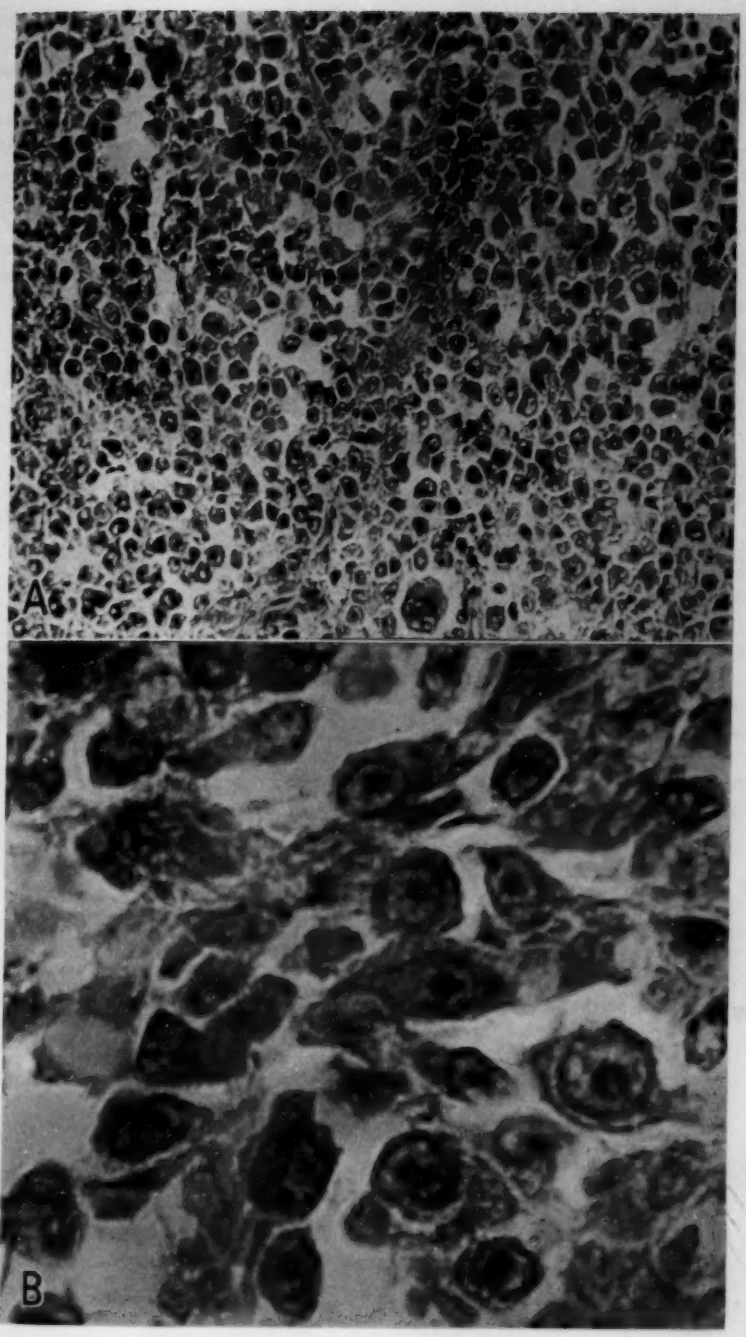


Fig. 2.—Primary endothelioma of the spleen—microscopic appearance: A, × 100, B, × 430.

*Histologic Examination.*—On microscopic examination, an area of spleen not invaded by tumor showed a marked decrease in the number and size of the malpighian corpuscles. A few normal lymphocytes were scattered about thickened arteries, but not in the usual structure of a corpuscle. The capsule was somewhat thickened; the pulp showed areas of increased fibrosis and diminution of cellular elements, some of which stained poorly. The sinusoids were dilated and contained a few red blood cells. Adjacent to this area, the sinusoids were compressed, and among them was seen an increased amount of fibrous tissue. The transition from splenic pulp containing no tumor tissue to the area of tumor cells was rather sharp. The cells of the tumor were large and polygonal or spindle shaped, with acidophilic cytoplasm. The nuclei were generally oval, occasionally lobulated, and vesicular, with deeply staining chromatin material and prominent nucleoli. Mitotic figures were fairly numerous. The size of the cells varied greatly, as did also the amount of their cytoplasm. The connective tissue was notably diminished. No normal splenic pulp was seen in this area. An occasional well formed sinus traversed this area, and it was seen that the endothelial cells lining the sinus greatly resembled the cells of the tumor tissue. The degree of anaplasia was striking throughout the section.

Other sections were similar in character, some revealing necrotic tissue, others displaying giant cells, the nuclei of which were identical in nature with those of the tumor cells.

Sections stained by the Foot method showed a slight amount of reticular structure in the tumor areas.

It seems possible that the tumor cells arose from the endothelium of the sinuses of the spleen.

Sections of the splenic flexure of the colon adjacent to the perforation of the intestine and to the splenic tumor showed necrosis of the serosal surface with an acute inflammatory reaction beneath. Just outside the intestine and lying in a fatty tissue stroma was a small area of lymph node completely replaced by tumor cells. A section of the stomach revealed changes similar to those seen in the intestine.

SUMMARY

An instance of primary endothelioma of the spleen with metastases only to the regional lymph nodes is reported. The patient suffered from marked secondary anemia, progressive cachexia and, late in the course of the disease, from pain in the upper part of the abdomen. Death occurred following peritonitis as a result of necrosis and perforation of the colon adjacent to the tumor.

# General Review

## ROLE OF VITAMIN B IN RESISTANCE

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CONTENTS



From the Laboratory Division of Montefiore Hospital.

CONTENTS—*Continued*



## I. INTRODUCTION

The existence in rice polishings of a substance that prevents beriberi in pigeons was established through the observations of Eijkman,[1] Grijns[2] and others. It was recognized that complete deprivation of so-called vitamin B led to polyneuritis, which was associated with anorexia, asthenia and atrophy of the visceral organs, with the exception of the adrenals, or to beriberi, which was characterized by polyneuritis and in addition, cerebellar symptoms, dilatation of the heart, edema and ascites.

But knowledge of vitamin B has grown in recent years, and now this vitamin is recognized to be a complex of several independent or interrelated factors. At the time of writing, it has been subdivided into several factors, though not all are acceptable.

Vitamin $B_1$ not only has been isolated and crystallized but has been synthesized, and there is no question that a deficiency of this factor of the vitamin B complex leads to polyneuritis and in most animals is essential to growth.

Vitamin $B_2$, or G, comprises several factors. One is the antipellagra factor that is important in the maintenance of a healthy condition of the skin. Another is $B_6$, a deficiency of which in rats leads to a specific dermatitis analogous to pellagra in human beings. A third factor is riboflavin, a deficiency of which in rats leads to alopecia, conjunctivitis,

1. Eijkman, C.: Geneesk. tijdschr. v. Nederl.-Indië **30**:295, 1890; Virchows Arch. f. path. Anat. **149**:187, 1897.

2. Grijns, G.: Geneesk. tijdschr. v. Nederl.-Indië **41**:3, 1901.

keratitis and cataracts. Vitamin $B_2$ (or G) complex may contain the extrinsic factor essential in the formation of the hemopoietic principle.

Vitamin $B_3$ has been described by Williams and Waterman [3] as essential for growth in pigeons. Carter and O'Brien [4] defined vitamin $B_3$ as a factor required for full restoration of weight in a pigeon on a diet which, in addition to vitamins $B_1$ and $B_2$, includes an adequate supply of basal dietary constituents, such as salt and protein. They reported that at least two factors appeared to be involved in vitamin $B_3$: one which is adsorbed from liver concentrates on fuller's earth, carrying with it riboflavin; the other present in the filtrate after treatment with this reagent.

Vitamin $B_4$, described by Reader [5] as essential for growth and normal health in rats, and vitamin $B_5$, of Carter, Kinnersley and Peters, to which a similar function is attributed, are not well established entities, nor has their existence been entirely confirmed.

Some investigators have regarded the manifestations of so-called vitamin $B_4$ deficiency in the rat as merely a chronic $B_1$ hypovitaminosis, which may be cured by giving sufficient quantities of vitamin $B_1$ (Kinnersley, O'Brien and Peters [6]; Waterman and Ammerman [7]).

Elvehjem and his co-workers [8] have expressed the belief that vitamin $B_4$ is a separate entity required for normal mammalian nutrition. By employing a ration more highly purified than the usual "vitamin B-free" diets they were able to produce the typical symptoms described by Reader [5] without first inducing the syndrome of vitamin $B_1$ deficiency. They showed that the symptoms persisted even after the administration of crystalline vitamin $B_1$ but disappeared when vitamin $B_4$ supplements were given. Chicks are said to be more susceptible to vitamin $B_4$ deficiency than are rats.[8]

A water-soluble vitamin present in liver extract and yeast which is distinct from the known vitamins was described by Elvehjem, Koehn and Oleson [9] and Frost and Elvehjem.[10] Rats placed on a purified diet in which vitamin $B_1$ was supplied in crystalline form, vitamins $B_4$ and $B_6$ from white corn and riboflavin and the antipellagra factor in con-

3. Williams, R. R., and Waterman, R. E.: J. Biol. Chem. **78**:311, 1928.

4. Carter, C. W., and O'Brien, J. R.: Biochem. J. **30**:43, 1936.

5. Reader, V.: Biochem. J. **23**:689, 1929.

6. Kinnersley, H. W.; O'Brien, J. R., and Peters, R. A.: Biochem. J. **29**:701, 1935.

7. Waterman, R. E., and Ammerman, M.: J. Nutrition **10**:35, 1935.

8. Kline, O. L.; Bird, H. R.; Elvehjem, C. A., and Hart, E. B.: J. Nutrition **11**:515, 1936; **12**:455, 1936. Kline, O. L.; Elvehjem, C. A., and Hart, E. B.: Biochem. J. **30**:780, 1936.

9. Elvehjem, C. A.; Koehn, C. J. J., and Oleson, J. J.: J. Biol. Chem. **115**: 707, 1936.

10. Frost, D. V., and Elvehjem, C. A.: J. Biol. Chem. **121**:255, 1937.

centrates prepared from liver extract failed to grow until the entire liver extract or yeast was added. The factor can be separated from liver extract by treating a concentrated aqueous solution of the powdered tissue with large volumes of a mixture of alcohol and ether. They termed this vitamin factor W. It is fairly stable and is not destroyed by boiling for short periods in strongly acid or alkaline solutions ranging from $p_H$ 1 to $p_H$ 9. It is likely that factor W is complex, and it seems to be different from the nicotinic acid amide described by Funk and Funk.[11]

Vitamin B (Water-Soluble B)

Vitamin $B_1$—Antineuritic Factor

Vitamin $B_2$ (or G)

P-P—Antipellagra factor (Goldberger[12])

G—Riboflavin, preventnig alopecia and cataracts in rats ($B_2$ proper)

$B_3$—Growth factor in pigeons (Williams and Waterman[3])

$B_4$—Growth factor in rats and chicks (Reader[5])

$B_5$—Growth factor in pigeons (Carter, Kinnersley and Peters), unconfirmed

$B_6$—Specific antidermatitis factor in rats (Györgyi[13])

W—Growth factor in rats and chicks distinct from $B_1$, $B_4$, $B_6$, riboflavin and P-P (Elvehjem, Koehn and Oleson[9])

Throughout this discussion, the term "vitamin B" refers to the complex; likewise, the term "vitamin $B_2$ (or G)" refers to the complex. When the individual factors have been specifically controlled, the terms "vitamin $B_1$," "vitamin $B_3$" and others are used.

An evaluation of the literature on vitamin B and particularly that dealing with the relation of the vitamin B factors to resistance is approached with some trepidation. Much of the latter can be accepted only with reservation and caution. A common error, particularly of the earlier investigators, was in assuming that when beriberi or polyneuritis developed in an animal that was being fed a diet of polished rice the animal was suffering solely from a deficiency of the antineuritic factor. Obviously, in the circumstances the animal was suffering from complete avitaminosis, from the effects of an extremely unbalanced diet, deficient in proteins, fats and mineral constituents, and from starvation. Interpretation of the resistance of such an animal to an induced infection is impossible. As knowledge of dietetics, of the vitamins and, finally, of their complexity was attained, experimental work in this field was better

11. Funk, C., and Funk, I. C.: Proc. Am. Soc. Biol. Chem. **8**:35, 1937.

12. Goldberger, J.: Medicine **5**:79, 1926.

13. Györgyi, P.: Nature, London **133**:498, 1934; Biochem. J. **29**:741, 1935.

controlled. But even in these experiments, the effects of undernutrition and starvation and of incidental spontaneous infection were usually not controlled.

In observations on human beriberi the most significant clinical feature is the result of a deficiency of vitamin $B_1$, but the nature of the diet suggests that other deficiencies in nutrition must be involved. Conclusions drawn from clinical experience on the incidence of infection in vitamin B deficiency are of necessity largely inferential and at best are a stimulus for experimental verification.

In spite of these obvious difficulties, a careful analysis of the literature in this field suggests strongly that vitamin B plays an important role in the natural resistance of the organism to infection. It is probable that the significance of vitamin B in the mechanism of resistance to infection is indirect and secondary to its importance in cellular metabolism and the processes of oxidation.

Empirical, as well as experimental, evidence indicates that relative deficiency of vitamin B has played an important role in the spread of infectious diseases among national and racial groups in the past and still does.[14]

## II. EFFECT OF DEFICIENCY OF VITAMIN B ON THE FORMATION OF NATURAL ANTIBODIES

Zilva [15] observed no effect of a vitamin B-deficient diet on the complement titer of the serum of rats.

Findlay and MacKenzie [16] studied the opsonic activity of the serum of rats fed a diet deficient in vitamin B. This diet consisted of caseinogin, starch, cod liver oil and inorganic salt mixture. Control rats were given a complete diet. After three and six weeks the serum removed from the rats was pooled in each group, and the opsonic activity tested against Bacterium coli and Staphylococcus aureus. The average number of bacteria phagocytosed per leukocyte was taken as the phagocytic index. No difference in the opsonic activity of the serum of the vitamin B-deficient animals was observed.

Using the Wright method of determining opsonic activity, Werkman,[17] in experiments in vivo, likewise observed no effect on the opsonic power of the serum of rats fed a vitamin B-deficient diet during a period of eleven weeks.

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14. For recent reviews on the general subject of vitamins and resistance see E. C. Robertson (Medicine **13**:123, 1934) and S. W. Clausen (Physiol. Rev. **14**: 309, 1934).

15. Zilva, S. S.: Biochem. J. **13**:172, 1919.

16. Findlay, G. M., and MacKenzie, R.: Biochem. J. **16**:574, 1922.

17. Werkman, C. H.: J. Infect. Dis. **32**:247, 1923.

Each rat was given an intraperitoneal injection of 1 cc. of a 20 per cent peptone solution twelve hours prior to the test. At the time of the test, each was given an injection of 1 cc. of an eighteen hour culture of Bacterium typhosum. After fifteen minutes, smears of the exudate were made by means of a Wright pipet following puncture of the peritoneum. The ratio of leukocytes to bacteria was determined by examining the stained smear. The average number of bacteria ingested per leukocyte was taken as the opsonic index. No difference in opsonic activity was noted in the rats fed a deficient diet as compared with those fed a normal diet. The in vitro test for opsonic activity likewise showed no significant alteration.

Incidental observations on the bacteriotropins for pneumococci in pigeons deprived of vitamin B were made by Findlay and Mackenzie.[16] The pigeons were fed a diet of polished rice and water for twenty days. One cubic centimeter of a culture of pneumococcus was injected intraperitoneally, and one, three, six and twelve hours later samples of the exudate were removed and films stained. The phagocytic index showed no essential difference in the pigeons with beriberi as compared with normally fed pigeons.

Findlay and MacLean [18] in subsequent observations noted in 9 rats fed a diet of caseinogen, starch, cod liver oil and inorganic salt mixture for a period of from twenty-five to forty days a drop in the bactericidal power of the whole blood for Staph. aureus. The bactericidal power was estimated by the slide cell method of Wright, Colebrook and Storer. The normally fed rats showed an average index of 70 per cent; the vitamin B-deficient rats, an average index of 43 per cent.

This observation is consistent with the experimental and clinical experience of Osawa,[19] who pointed out that pigeons with beriberi show striking susceptibility to Staph. aureus. In such birds multiple abscesses of muscles develop, analogous to the acute purulent polymyositis seen in patients with mild beriberi. (See section on polymyositis, page 552.)

SUMMARY

In rats deficient in vitamin B the bactericidal power of the blood for Staph. aureus may be diminished. The titers of natural antibodies for other bacteria in rats and pigeons are apparently uninfluenced by this deficiency. More accurate data on this matter are desirable.

## III. EFFECT OF DEFICIENCY OF VITAMIN B ON THE FORMATION OF ACQUIRED ANTIBODIES

Rats fed a diet deficient in vitamin B but adequate in other respects showed no decrease in their capacity to produce hemolysins for sheep cells. Zilva [15] studied the formation of agglutinins for Bact. typhosum,

18. Findlay, G. M., and MacLean, I. S.: Biochem. J. **19**:63, 1925.

19. Osawa, Y.: Beitr. z. klin. Chir. **146**:621, 1929.

as well. Five rats fed a diet deficient in vitamin B for several weeks were given three injections of a culture of Bact. typhosum (killed by heating to 60 C. for one-half hour) at intervals of seven days. The agglutinin titers were determined one week after the last injection. The titers showed great variation, but none of the animals on the deficient diet produced as much agglutinin as those on an adequate diet. In repeating the experiment, no significant differences in the two groups were noted.

Werkman [17] confirmed the observations of Zilva.[18] He studied the influence of a vitamin B-deficient diet on the production of specific agglutinins and bacteriotropins for Bact. typhosum in rats and pigeons.

The diet of the rats consisted of casein 18 per cent, dextrin 74 per cent, salt 5 per cent and butter fat 3 per cent. The deficient pigeons were fed polished rice, and the control pigeons, a diet of white corn 65 per cent, linseed oil meal 3 per cent, ground oats 22 per cent, casein 5 per cent, tankage 3 per cent, calcium carbonate 1 per cent and sodium chloride 1 per cent. Of 13 rats kept on the vitamin B-deficient diet during a period of thirteen weeks, 7 died before tests were made. The remaining 6, together with 5 normally fed animals, were each given 0.3 cc. of an eighteen hour broth culture of Bact. typhosum, and another 0.3 cc. after an interval of seven days. After the second injection, they were bled. No significant difference in the titers was observed. The experiment was repeated, with a similar result.

In another series of experiments, Werkmen [20] fed 6 rats on the vitamin B-deficient diet during a period of twelve weeks. These, together with 6 controls, were each given two intraperitoneal injections of 0.2 cc. of an eighteen hour broth culture of Bact. typhosum at a six day interval. Seven days after the last injection, the bacteriolytic power of the pooled serum was determined by the plate culture method, colony counts being made after twenty-four hours' incubation. No difference in the bacteriologic titers of the serums of the two groups was observed.

Thirteen pigeons were fed polished rice, and each received 0.1 cc. of a killed eighteen hour culture of Bact. typhosum culture on the eighteenth day intraperitoneally, 0.3 cc. on the twenty-sixth day and 0.5 cc. on the thirty-third day. Only 3 birds survived. The agglutinin titers of the serums of these birds were the same as those of the controls (Werkman [20]).

Apparently, then, vitamin B deficiency in the rat and in the pigeon is not associated with depression in the capacity to produce specific agglutinins or bacteriolysins for Bact. typhosum.

However, Guerrini [21] found that pigeons fed a diet of polished rice lose their capacity to produce agglutinins for Bact. coli.

He gave pigeons that were fed polished rice and water for from nine to ten days injections of a suspension of a killed culture of Bact. coli. The culture was given in increasing amounts every other day for eight injections. The birds were

20. Werkman,[17] pp. 255 and 263.

21. Guerrini, G.: Ann. d'ig. **31**:597, 1921.

bled and the titers determined after the fourth and after the eighth injection. The average titer of 3 pigeons fed on whole rice varied from 1:800 to 1:1,200 after the fourth injection and rose to between 1:7,000 and 1:9,000 after the final injection. The average titer of the 3 pigeons with beriberi was from 1:25 to 1:50 after the fourth injection and from 1:100 to 1:150 after the last.

As in many experiments in this field, the birds Guerrini observed were fed a diet completely deficient in all the vitamins and not only in vitamin B. This makes his observations consistent with the observations of Blackberg,[22] who, in an undetailed report, observed that rats fed a diet free from all vitamins show a decrease in the capacity to produce agglutinins and bacteriolysins.

It is interesting that with large amounts of living organisms as antigen the differences were less significant. Blackberg [22] believed that such cultures furnish a supply of vitamins. This may account for some of the contrary observations of Werkman [20] and Guerrini. Guerrini [21] used dead organisms as antigen in studies of the production of agglutinins and observed depression in the formation of antibodies. Werkman [20] used living cultures which may have supplied vitamin B in part and therefore negated the conditions of the experiment.

SUMMARY

Investigations bearing on the question whether a diet deficient in vitamin B may lead to depression in the capacity to form specific antibodies have produced contradictory results. There is, however, a preponderance of evidence that neither rats nor pigeons show decrease in their capacity to produce agglutinins for Bact. typhosum. Two factors may account for the contradictory results: 1. If living cultures were used as the source of the antigen, the living organisms may have supplied vitamin B to the deficient animal. 2. Much of the work was done in pigeons deficient in all vitamins. When the dietary conditions were somewhat better controlled, a deficiency of vitamin B did not impair the capacity of rats to form specific antibodies.

## IV. EFFECT OF DEFICIENCY OF VITAMIN B ON ANAPHYLAXIS

Abderhalden and Wertheimer [23] demonstrated increased susceptibility to anaphylaxis in pigeons fed polished rice. Four birds were fed a deficient diet; four, a normal diet. Each bird received one intraperitoneal injection of cattle plasma and twenty days later another. Three of the normally fed animals survived the second injection; the

22. Blackberg, S. N.: Proc. Soc. Exper. Biol. & Med. **25**:770, 1928.

23. Abderhalden, E., and Wertheimer, E.: Arch. f. d. ges. Physiol. **196**:440, 1922.

pigeons on a deficient diet all showed a marked drop in temperature and died. The authors attribute the severity of the reaction to the depression in cellular metabolism induced by the deficiency and increased by anaphylactic shock. Here as well, the effects induced by complete avitaminosis and starvation were not controlled.

Normally fed rats cannot be sensitized by injections of protein, and it is difficult or impossible to induce anaphylactic shock in them. Longcope [24] associated this difficulty with the low level of precipitin in the serum. However, Wedgewood and Grant [25] observed that with deficiency of vitamin B in the food of young rats it is possible to sensitize them to foreign protein so that they may be thrown into fatal anaphylactic shock on the second or third injection of the protein. It is interesting that vitamin A deficiency is without effect in this regard.

In their studies, the normal diet contained casein, rice, hydrogenated cottonseed oil, butter, salts and vitamin B as yeast. Of 5 normally fed rats given two injections each of 1 cc. of a 2 per cent solution of egg albumin intraperitoneally at an interval of two weeks, none died. Of 5 rats fed the same diet without yeast, all died under similar conditions. All the normally fed rats received the same number of injections, but none succumbed.

It was further observed by these investigators that it was possible with an injection of protein to produce death in young rats when these were on a complete diet if the rats were suffering from an infectious disease of the lungs at the time of the injection. Such rats apparently acquired sensitivity to foreign protein similar to that produced by deficiency of vitamin B, and it was suggested that this disease exhausted the reserve of vitamin B.

They concluded that in the rat vitamin B either desensitizes, prevents sensitization or protects against anaphylactic shock. The experiments are suggestive, and their importance warrants careful confirmation. It is well known that many nonspecific conditions, particularly infections, will increase the sensitivity of animals to anaphylactic shock. In animals suffering from deficiencies of vitamin B, A or C, spontaneous concomitant infections are frequently present and in studies of this type must be rigidly excluded. It is probable, however, that deficiency of vitamin B increases the sensitivity of certain animals to anaphylaxis.

It may be that in human beings the enormous variability in allergic response to proteins is in part due to variation in the deficiency of vitamin B in the dietary. This problem requires further investigation, and the investigation may lead to observations of clinical significance.

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24. Longcope, W. T.: J. Exper. Med. **36**:627, 1922.

25. Wedgewood, P. E., and Grant, A. H.: M. Bull. Univ. Cincinnati **2**:172, 1924.

## V. EFFECT OF DEFICIENCY OF VITAMIN B ON NATURAL RESISTANCE TO SPONTANEOUS INFECTIONS

### SPONTANEOUS INFECTIONS IN ANIMALS

There is some confusion in the literature as to the effect of deficiency of vitamin B on resistance to infection. This springs from the fact that only in recent years has it been possible to separate the various factors in vitamin B.

*Salmonella Suipestifer in Pigeons.*—McCarrison [26] succeeded in obtaining Salmonella suipestifer in pure culture from the blood and organs of 20 pigeons fed a diet of polished rice for a sufficient period to produce polyneuritis. In a subsequent experiment, 24 pigeons were isolated in separate cages. Twelve were fed polished rice, and 12, a diet of mixed grains. Those fed polished rice died in from nine to fifteen days. Two died of starvation on the ninth and twelfth days, respectively. The blood and internal organs were sterile and the sciatic nerves normal. One died in fifteen days, and the culture of its blood was positive for S. suipestifer. McCarrison gave the 12 normally fed pigeons injections of the culture of S. suipestifer. Of these, 9 died within forty-eight hours, and 3 survived for from three to twenty-three days. It is interesting that in those surviving for five days or longer peripheral polyneuritis developed although their diet had been complete. This was probably due to the diarrhea and interference with digestion. Similar results were observed in 12 other normally fed pigeons.

With this organism, McCarrison [26] reported that he produced some paralysis of the hindlegs in rabbits that were inoculated with the culture and survived for a period longer than two days.

The implication that this organism played an etiologic role in the development of polyneuritis was not substantiated, and the attempt at protection of the fowl with attenuated cultures of the organism against a subsequent dietary polyneuritis failed.

The observations, however, indicate the susceptibility of animals with beriberi to spontaneous infection with S. suipestifer. However, the pigeons were given diets deficient in vitamins other than vitamin B. This unfortunate assumption that a fowl suffering from beriberi when fed a diet of polished rice is deficient only in vitamin B recurs with astonishing frequency in the literature even up to very recent years.

In studying the anemia associated with beriberi in pigeons, Barlow [27] observed that a type of anemia occurs which suggests that some hemolytic agent is concerned with the genesis of the blood changes. There was relative hydremic plethora; the color index was more than 1. He attempted to correlate the anemia with bacterial invasion and observed

26. McCarrison, R.: Indian J. M. Research **6**:275, 1918-1919.

27. Barlow, O. W.: Am. J. Physiol. **93**:161, 1930.

a greater degree of bacteremia in pigeons fed a vitamin B-deficient diet than in those fed an adequate diet. The extent of the invasion of the blood paralleled closely the severity of the subsequent anemia. The bacterial invasion, he believed, was secondary to the dietary deficiency. Such changes were not due to the diet of polished rice alone, for they occurred in pigeons fed a purified complete diet free only from vitamin B. He unfortunately gives no details concerning his data, the organism isolated, the number of animals observed or the severity of the anemia.

*Virus of Epithelioma Contagiosum in Pigeons.*—McCarrison [28] described the appearance of wartlike nodules (epithelioma contagiosum) the size of a pea or larger in fowl deprived of vitamin B. These nodules appeared where feathers were few or absent, i. e., about the nostrils, openings of the ears, comb and wattles, and also in the buccal mucous membranes. It has been suggested that epithelioma contagiosum of fowl is due to infection with a virus. The virus exists in the blood, internal organs and fluids expressed from the lesions and is resistant to heat. It is readily transferred by rubbing the juice of the lesions into the skin.

McCarrison observed that the occurrence of epithelioma contagiosum in pigeons is favored by faulty nutrition and deranged metabolism. He produced polyneuritis in a number of pigeons by feeding them a diet of washed milled rice. Twelve were cured at the height of the disease by giving them artificial feedings of green mongo (an Indian plant used for food). Forty-eight hours after the disappearance of the symptoms of polyneuritis, these pigeons were placed with 60 healthy stock birds, fed on an adequate stock diet. Within a period of from six weeks to three months, 7 of the 12 cured birds spontaneously acquired epithelioma contagiosum, while all of the 60 normally fed birds remained well. The growths had the usual dissemination. It is interesting that emulsified material of the nodules rubbed into scarified surfaces at the angles of the mouths of 6 healthy pigeons did not produce new growths in any. The virus was not so virulent as to infect normally fed birds but was sufficiently virulent to infect poorly nourished ones.

It must be mentioned again that while polyneuritis is due to absence of vitamin B, the disorder in metabolism that follows the feeding of a diet exclusively of polished rice is a consequence of a deficiency not only of vitamin B but of other vitamins as well, and of proteins, fats and inorganic salts. The diet contains, further, an excess of starch.

McCarrison [28] pointed out that complete avitaminosis inhibits growth of tumors but that incomplete avitaminosis may favor their growth. From McCarrison's experiments, it is suggested that transient severe deficiency of vitamins favors growth of the virus of epithelioma contagiosum and alters the susceptibility of the tissues to its presence.

28. McCarrison, R.: Brit. M. J. **2**:172, 1923.

*Gastrointestinal Infections and Diseases in Monkeys and Rats.*—McCarrison [29] maintained that in India deficiency of vitamins in the food, particularly of vitamin B, is an important factor in the causation of gastrointestinal disorders and infections, mucous disease in children and colitis in adults. To test this hypothesis, he placed wild monkeys on a diet partially deficient in vitamins, particularly in vitamin B. The control animals were fed a diet of whole meal, bread, milk, ground nuts, onions, fresh butter, plantains and water. The deficient diet was the same as the control diet, but the food was autoclaved. Fresh onion was given, however, for vitamin C and some fresh butter for vitamin A. The animals fed the control normal diet were maintained in good health. Those given the deficient diet survived for from fifty-one to one hundred days. They lost weight, their appetite was poor, and they began to have diarrhea and dysentery. Endamoeba histolytica was found in the stools, and active forms were present. Of 8 controls, 2 were found to be carriers of the endamebas. At autopsy, dilatation of the stomach was marked; congestion of the gastrointestinal tract and peritoneal ecchymoses were present together with colitis in varying degrees. Histologically, he observed atrophy of the musculature of the bowel, particularly in the colon, degenerative changes in the myenteric plexus of Auerbach, atrophic and necrotic inflammatory changes in the mucous membrane and bacteria in all coats of the intestinal wall. These changes were most pronounced in the 6 monkeys given the diet deficient in both vitamins A and B and in 2 of 5 monkeys given a diet deficient only in vitamin B.

Rats fed on a diet deficient only in vitamin $B_1$ show a high incidence of gastric ulcer (Dalldorf and Kellogg [30]).

Sixty-four rats of the same stock were fed Sherman-Spohn diet 107, which is deficient in vitamin B and in vitamin C, for forty days. Vitamin G (or $B_2$) was supplied in the form of large amounts of autoclaved yeast. Twenty additional rats were kept on a complete dietary, and six were given small amounts of vitamin B for from fifteen to fifty-four days. In the last two groups, none acquired gastric lesions. Seventy-three per cent of the group fed diets deficient in vitamin B were found to have ulcerations of the gastric mucosa. Of these, 8 had chronic indurated ulcers, resembling chronic peptic ulcers in man. In 47, the lesions were acute erosions. The chronicity of the ulcers seemed to relate to the duration rather than to the degree of deficiency. The lesions were located along the lesser curvature of the stomach and were comparable to the lesions in the human stomach.

The importance of vitamin $B_1$ as a factor in growth has been well established. The effect of lack of vitamin $B_1$ is a striking cessation of appetite. This decrease in appetite leads to decrease in consumption of food. With the addition of vitamin $B_1$ improvement in appetite

29. McCarrison, R.: Proc. Roy. Soc. Med. **18**:3, 1925.
30. Dalldorf, G., and Kellogg, M.: J. Exper. Med. **56**:391, 1932.

occurs, with increase in consumption of food and therefore in growth of the young. In this sense, vitamin $B_1$ is a growth-promoting factor. Loss of weight in deficiency of vitamin $B_1$ is not a symptom characteristic of the deficiency but a symptom of loss of appetite from other causes, i. e., of simple starvation (Sherman and Smith [31]).

An inhibitory effect exerted specifically by deficiency of vitamin B on the secretion of certain substances by the gastrointestinal tract, such as secretin, has not been substantiated experimentally (Cowgill and Gilman [32]), for in the intestinal mucosa from polyneuritic dogs secretin has been demonstrated.

The observations of McCarrison [28] on the loss of motility of the intestinal tract were confirmed experimentally by Gross in rats deficient only in vitamin B, and he expressed the opinion that vitamin B probably plays an important part in the intestinal stasis encountered in everyday life. Marked gastric atony occurs in experimental deficiency of vitamin B, and Cowgill, Deuel, Plummer and Messer [33] found that this is consistent with similar clinical observations in human beriberi.

These observations suggest that disturbances analogous to peripheral neuritis occur in the nerves innervating the stomach, and that such degenerative processes may lead to trophic changes in the gastric mucosa and disturbances in intestinal motility. Whether secondary infection and trauma are important in the pathogenesis of these lesions is not established.

*Cutaneous Infections in Dogs.*—Cowgill, Stucky and Rose [34] observed infections of the skin and other cutaneous manifestations related to deficiency of vitamin B in dogs. The diets contained either casein or meat residue and sucrose, lard, butter, bone ash and salt mixture. These animals presented symmetrically placed round or oval lesions over the bony prominences on the flexor and exterior surfaces of the forelimbs and hindlimbs. In the early stages, the lesions appeared as slightly eleveated pink areas denuded of hair, which gradually sloughed away. Punched-out ulcers with sharp edges and red bases, free from exudate, resulted. Administration of vitamin B checked further development of the lesions and encouraged healing. The absence of gross manifestations of tenderness, excessive heat and local congestion suggested the nutritional character of the lesions. The relation of these lesions to those observed in deficiency of vitamin $B_6$ (antidermatitis factor in rats) and riboflavin should be determined.

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31. Sherman, H. C., and Smith, S. L.: The Vitamins, New York, The Chemical Catalog Company, Inc., 1931.

32. Cowgill, G. R., and Gilman, A.: Arch. Int. Med. **53**:58, 1934.

33. Cowgill, G. R.; Deuel, H. J., Jr.; Plummer, M., and Messer, F. C.: Am. J. Physiol. **77**:389, 1926.

34. Cowgill, G. R.; Stucky, C. J., and Rose, W. B.: Arch. Path. **7**:197, 1929.

SPONTANEOUS INFECTIONS IN MAN

There are numerous observations in clinical medicine of the effect of vitamin B deficiency on resistance to infection. The concomitant prevalence of dietary deficiency and infections that reach endemic proportions in certain Asiatic countries is striking. Analysis of the diet utilized by large sections of the population suggests a low content of vitamin B as well as other partial deficiencies.

Of particular importance is the occurrence of leprosy. It has been shown in recent work that continued maintenance of rats on a diet deficient in vitamin $B_1$ leads to progressive increase in their susceptibility to leprosy. An analysis, however, of the deficiencies responsible for the general low racial resistance to numerous infections in the Orient is extremely difficult, if not impossible. That deficiency of vitamin B is one of the most prominent contributory causes cannot be denied.

*Polymyositis.*—The spontaneous occurrence of polymyositis with abscess formation in patients suffering from deficiency of vitamin B and latent beriberi was observed by Osawa [19] in Japan. He reported 30 instances of purulent myositis occurring in a six month period between January and October 1927, in patients ranging in age from 1 to 50 years. Most of the patients were between 21 and 30 years of age. The purulent nodules were multiple, and pure cultures of Staph. aureus were obtained in 18 cases. Other symptoms included hypoesthesia or hyperesthesia, patchy or diffuse, and in more than half of the cases so-called calf pain. These were considered early manifestations of vitamin B deficiency. Apparently, 13 patients had definite signs of beriberi; only 4 showed no signs or symptoms of this disease. Osawa [19] extended these studies experimentally and demonstrated the frequency of purulent myositis in vitamin B-deficient animals given injections of cultures of Staph. aureus. (See part VI, page 555.)

The increased susceptibility of patients with latent and manifest beriberi to staphylococcic infection probably accounts for the frequency of polymyositis in Japan, where a diet of rice is widespread.

Though the observations made clinically on the deleterious effects of latent and manifest beriberi are extensive and suggest the important role of vitamin B in metabolism, nutrition and the resistance of certain tissues to infection, it must again be emphasized that the relationship is not clear when viewed only from the standpoint of the clinical data. For, in the tropics, though inadequate diets are prevalent, such diets are insufficient not only in vitamins but in proteins and calories as well.

*Infections of Nose and Ears.*—In investigating the etiologic role of vitamin deficiency in otolaryngologic infections, Cody [35] studied the

35. Cody, C. C.: Arch. Otolaryng. **16**:661, 1932.

histologic changes of the nose, ears and accessory sinuses in animals fed on a diet deficient in vitamin B. Rats were placed on the diet for three or four months, and the survivors were restored to a normal diet for four additional months. Ten were fed on a diet deficient in vitamin $B_1$, 12 on a diet deficient in vitamins $B_1$ and $B_2$ and 10 on a diet deficient in vitamin $B_2$. A deficiency of vitamin $B_2$ (or G) produced no abnormalities in the ears, nose or throat. The group deficient in vitamin $B_1$ showed a polycystic appearance of the nasal mucosa in the upper postethmoid region; this was due to dilation of the lumens of Bowman's glands and fattening of the lining membrane of cuboid cells.

Clinically, the author believed, the deficiency of vitamin B was associated with atrophy of the lymphoid tissue and leukopenia. He attributed to the vitamin B deficiency aphthous ulcers and gingivitis with postnasal discharge. The posterior tips of the turbinates were smooth, atrophic, moist and creamy white. Additions of yeast to the diet relieved these conditions. Degenerative changes in the auditory nerve and in the ganglion cells of the vestibular nerve were also observed.

It is possible that vitamin B deficiency associated with atrophy of the lymphoid tissue of the nasopharynx and atrophic changes in the mucosa may result in increased susceptibility of these tissues to infection.

*Polyneuritis and Infection.*—There is a group of diseases clinically termed polyneuritis which many neurologists believe to be associated with deficiency of vitamin B.[36] The polyneuritis of alcoholism is due not to the effects of alcohol, but to inadequate nutrition, i. e., to the interference with digestion and assimilation secondary to alcoholism. It has been shown that a high intake of vitamins, particularly of vitamin $B_1$, even in the presence of continued consumption of alcohol, will lead to recovery.

The neuritis of diabetes mellitus is probably a disease due to deficiency of vitamin $B_1$, as the dietary recommended to patients with diabetes is obviously low in this vitamin. Cereal foods and certain fruits, such as bananas, fairly rich sources of vitamin B, are usually eliminated for years. Addition of a vitamin B concentrate is followed by definite improvement (Russell[37]; Theobald[38]).

Similarly, the neurologic disturbances associated with pernicious anemia and achylia may be secondary to deficiency of vitamin B.

The importance of polyneuritis in cutaneous infections of the extremities will be stressed later, but it is interesting at this point to

36. Wechsler, I. S.: Arch. Neurol. & Psychiat. **29**:813, 1933. Goodhart, R., and Jolliffe, N.: J. A. M. A. **110**:414, 1938.

37. Russell, W. R.: Edinburgh M. J. **43**:315, 1936.

38. Theobald, G. W.: Lancet **1**:834, 1936.

comment that in diabetic persons with arteriosclerosis the infections of toes that precipitate thrombosis and gangrene always occur in the leg that shows evidence of peripheral so-called diabetic neuritis. The high incidence of senile arteriosclerosis unassociated with gangrene as compared with the frequency of gangrene in the diabetic person with arteriosclerosis suggests a precipitating factor of diminished dermal resistance to infection in the person with diabetes. This is well known and accepted by surgeons, but the important role that the neuritic element plays is not so well recognized. The administration of vitamin B concentrates, even in the older diabetic person, is associated with improvement of the neuritis and may help to lower the future incidence of peripheral infection and gangrene.

SUMMARY

Pigeons suffering from manifest or latent beriberi show spontaneous development of infection with S. suipestifer. Artificial diets complete in all known respects save that of vitamin B reduce the natural resistance of pigeons to spontaneous bacterial infection. Epithelioma contagiosum, a tumor in fowl induced by a filtrable virus, occurs with great frequency in pigeons placed for a time on a diet of polished rice even though the birds are again given an adequate diet.

Gastric ulcers are frequent in rats fed diets deficient in vitamin $B_1$. Dogs show ulcerations and infections of the skin over the bony prominences of the legs, analogous to decubiti, when given diets deficient in vitamin B. The addition of this vitamin complex to the diet checks the development of the ulcers and encourages healing of the lesions.

Though further investigation in this field is desirable, there is evidence from observations on pigeons, rats and dogs that deficiency of vitamin B is associated with decrease in natural resistance to spontaneous infections. In human beings suffering from latent or manifest beriberi, infections are likewise frequent. In part this is associated with alteration in general or in cellular metabolism, and in part it is secondary to trophic disturbances precipitated by the degenerative process in peripheral nerves. Of course, in many instances, it is impossible to establish the responsible dietary factor.

In Japan, multiple purulent myositis with abscess formation in the muscles is apparently associated with vitamin B deficiency. This is attributed to the increased susceptibility of patients with beriberi to infection with Staph. aureus.

It has been suggested that certain nasal conditions, such as aphthous ulcers associated with atrophy of the mucosa, particularly over the turbinates, and disappearance of lymphoid tissue in the nasopharynx, may be secondary to vitamin B deficiency.

Clinically, the etiologic importance of vitamin B deficiency in the production of the so-called polyneuritis of alcoholism and diabetes mellitus has been established. It is suggested that the neuritis in diabetic persons, by inducing trophic changes in the skin, enhances the possibility of local infection and may be a precipitating factor in the production of gangrene.

Though requiring confirmation in more extended observation, scattered evidence reported in the literature indicates increased frequency of spontaneous infections in animals and man fed diets deficient in vitamin B. This is dependent not only on alterations in cellular metabolism but also on trophic changes secondary to the polyneuritis.

## VI. EFFECT OF DEFICIENCY OF VITAMIN B ON NATURAL RESISTANCE TO INDUCED INFECTIONS

Attempts to determine quantitatively the decrease in natural resistance by artificial induction of infections or by administration of toxic drugs in animals deficient in vitamin B are reported in a number of communications in the literature. The greater number of these reports do not stand critical analysis. Either the data are statistically inadequate or, more frequently, a deficiency of other dietary factors was permitted. The latter fault is noted particularly of work in which beriberi was induced in pigeons by the feeding solely of polished rice. Under such experimental conditions the results depict the effects of partial starvation rather than those of vitamin B deficiency alone.

### ACUTE INFECTIONS

*Staphylococcus Aureus.*—Recently Rose and Rose [39] observed the effect of deficiency of vitamin $B_1$ on the resistance of dogs to staphylococcic infection.

Sixteen dogs were fed an artificial diet complete in all known respects except that of vitamin $B_1$ (the casein III diet of Cowgill [40]). Of these, 8 animals served as controls, their diets being supplemented with vitamin $B_1$ in adequate amounts. The remaining 8 were rendered chronically deficient in vitamin $B_1$ by being fed a variable fraction of the minimal vitamin $B_1$ requirement. The concomitant caloric deficiency usually observed with B avitaminosis was avoided by restricting the quantity of food given the control dogs to that amount voluntarily consumed by the animals deficient in vitamin $B_1$. After a period of eighty-one days on the diet, all the dogs were given injections of washings of a twenty-four hour agar culture of Staph. aureus isolated from a human carbuncle. Each dog received between 1 and 2 billion organisms.

39. Rose, S. B., and Rose, W. B.: J. Infect. Dis. **59**:174, 1936.

40. Cowgill, G. R.: The Vitamin B Requirement of Man, New Haven, Conn., Yale University Press, 1935.

There was practically no difference in the incidence of survival in the two groups. However, of the surviving dogs, those that were fed a diet deficient in vitamin $B_1$ had positive blood cultures for a longer period of time than the control animals (twelve and two tenths and six days, respectively). In the first ten days after the infection was induced, the vitamin-deficient dogs lost twice as much weight as the controls. The authors concluded that animals rendered partially deficient in respect to vitamin $B_1$ appeared to be more susceptible to the deleterious effects of an induced infection with Staph. aureus.

*Erysipelothrix Rhusiopathiae.*[41]—Pigeons fed a diet of polished rice succumb readily to injections of a culture of Ery. rhusiopathiae (Setti[42]) in amounts that normally fed birds survive. If pigeons are immunized by repeated injections of such cultures during a period preceding the administration of the vitamin-deficient diet the resistance thus acquired is sufficient to prevent death of some of the animals following a subsequent injection of a quantity of the culture lethal for nonimmunized birds. However, the acquired resistance seems definitely diminished, since an occasional pigeon succumbed. The numbers of animals used were small. Setti[42] also noted that the longer an animal was given the diet of polished rice, the quicker it succumbed to the attack of the organism of swine erysipelas, the maximal drop being attained at twenty-one days.

This is obvious, since death from polyneuritis alone not infrequently occurs at an earlier date. However, somewhat unexpected is the fact that in pigeons fed a diet of polished rice for twenty-four days the natural resistance to Ery. rhusiopathiae returned to the normal level when the pigeons were given a normal diet for from twenty-four to forty-eight hours. The data on which this conclusion is based are not given. It is known that restoration to apparent health even after prolonged deficiency of vitamin B and even when treatment is initiated

41. Swine erysipelas is caused by a slender gram-positive bacillus, Ery. rhusiopathiae. It most frequently affects young animals. There is an acute form characterized by severe prostration, high fever, anorexia, thirst and conjunctivitis. Within twenty-four hours, bright red patches appear on the skin over the ears, snout, neck and abdomen. The mortality is over 80 per cent within four days. Mild chronic forms and arthritic forms occur. It may be transmitted to man. In pigeons normally fed 0.1 cc. of a twenty-four hour broth culture, inoculated intramuscularly, usually proves fatal in three or four days. Death is often preceded by paralysis of the legs, dyspnea and convulsions. Post mortem, a black hemorrhagic mass is found in the muscle at the site of inoculation. The spleen is enlarged, and there are often punctiform hemorrhages in the mucosa and viscera and an exudate in the pericardium (Crimi, cited by Topley, W. W. C., and Wilson, G. S.: The Principles of Bacteriology and Immunity, New York, William Wood & Company, 1932, vol. 2, p. 799).

42. Setti, C.: Biochim. e therap. sper. **9**:197, 1922.

almost at the point of death is dramatic and rapid. McCarrison [26] observed, however, evidence of impaired resistance months after the diet was restored to normal. Setti [42] reported that a diet of wheat, whole rice or maize caused the same drop in resistance as one of polished rice if the food was first autoclaved at high temperature.

The interesting observation was made in these studies that the attenuated strains of Ery. rhusiopathiae or of B. anthracis injected into pigeons with B avitaminosis increased in virulence to such a degree that they became lethal for normally fed animals.[43] Zinsser, Ruiz Castañeda and Seastone [44] observed enhanced growth of the rickettsias of typhus in guinea pigs fed an avitaminic diet.

*Diplococcus Pneumoniae.*—The natural resistance of pigeons fed polished rice to pneumococcic infection is markedly decreased (Findlay [45]). It is well known that normal pigeons are completely resistant to such infection, the injection of large numbers of pneumococci never being followed by clinical disease or death. Findlay [46] attributed the change in resistance to the reduction of body temperature incident to vitamin B deficiency.

Four pigeons were fed a diet of polished rice until the normal cloacal temperature of 106 F. was reduced to 103 F. This occurred after periods of from twenty-one to eighty-six days. These pigeons, together with 4 controls, were inoculated intraperitoneally with the washings of 6 blood agar slants of pneumococci. Death took place in the birds with beriberi from nine to thirty-six hours after they were inoculated. The controls presented no symptoms. The cardiac blood of the normally fed birds was sterile. No bird of the group with beriberi showed symptoms of paralysis either before or after the injection of pneumococci.

Findlay [45] reduced the body temperature of pigeons to 101 F. by subcutaneous injections of 0.46 Gm. of aminopyrine. The pigeons were then inoculated with pneumococci of type II (washings of 4 agar slants). The pigeons died, and pneumococci were recovered from the cardiac blood and organs. A diet of polished rice brings about a reduction in body temperature by direct action on the central nervous system or by retardation of all metabolic processes.

Similar observations were reported by Werkman,[20] who fed 5 pigeons an artificial diet deficient only in vitamin B. Following intraperitoneal injection of single 0.1 cc. doses of a twenty-four hour culture

43. According to W. W. C. Topley and G. S. Wilson (The Principles of Bacteriology and Immunity, New York, William Wood & Company, 1932, vol. 2, p. 799), most strains of Ery. rhusiopathiae are pathogenic for normal pigeons.

44. Zinsser, H.; Ruiz Castañeda, M., and Seastone, C. V., Jr.: J. Exper. Med. **53**:333, 1931.

45. Findlay, G. M.: Lancet **1**:714, 1922.

46. Findlay, G. M.: J. Path. & Bact. **26**:485, 1923.

of a strain of pneumococcus isolated from a rat, all died. Five controls all survived. The organism was highly virulent for mice.

*Neisseria Meningitidis (Cerebrospinal Meningitis).*—Further evidence of the effect of a vitamin $B_1$-deficient diet on resistance was reported by Findlay.[46] He inoculated 10 normally fed pigeons and 10 pigeons fed a diet deficient in vitamin B with 3,000 million bacteria of a strain of N. meningitidis (Diplococcus intracellularis-meningitidis). The normally fed controls all survived. Ten of the deficient birds, the temperature of which fell below 40 C. (104 F.), died, and 10 in which the temperature remained above 40 C. survived. The administration of aminopyrine to normal birds in amounts sufficient to reduce the temperature to 38 C. (100.4 F.) had an effect similar to that of vitamin $B_1$ deficiency in reducing their natural resistance to the meningococcus.

*Bacillus Anthracis.*—Rats and pigeons are naturally highly resistant to B. anthracis, but when fed a diet deficient in vitamin B these animals become highly susceptible to infection with this organism (Werkman [20]).

Eleven rats were given a diet of casein, dextrin, salt and butter fat for a period of two weeks. Together with 4 normally fed animals, they were then given single injections of 1.25 cc. of a suspension of a culture of B. anthracis intraperitoneally. The culture was known to kill a rabbit in forty-eight hours when 0.1 cc. of the suspension was administered. Of the deficient rats, 7 died and 4 survived. All the normally fed animals survived. In this experiment, not only was vitamin B omitted from the diet, but vitamin D as well.

Pigeons fed either a diet of polished rice or an artificial diet lacking only in vitamin B and given single intraperitoneal injections of 0.1 cc. of a suspension of a culture of B. anthracis all succumbed. Five birds were used in each group. Ten controls all survived.

*Bacterium Coli.*—The natural resistance of pigeons fed a diet deficient in vitamin B is lowered to Bact. coli. Findlay [46] reported that of 20 normally fed birds inoculated with 1,500 million colon bacilli intraperitoneally, only 2 succumbed to the induced infection and that of 12 vitamin B-deficient birds fed exclusively on polished rice, 9 died. He maintained that the critical factor was the drop in the temperature of the animal to a range that permitted growth of certain bacteria to which the pigeon is normally highly resistant. Pigeons given doses of aminopyrine showed a similar drop in resistance. Here, again, complete avitaminosis was induced.

*Bacterium Enteritidis (Gaertner).*—Of 10 pigeons fed a diet of polished rice for a period of twenty days and inoculated with 2,000 million organisms of a strain of Bact. enteritidis (Gaertner), all succumbed to the infection. Only 8 of 20 normally fed animals similarly inoculated died (Findlay [46]).

The effect of diets deficient only in vitamin B on the natural resistance of young albino rats to Bact. enteritidis (Gaertner) was determined by Verder.[47]

The rats were from 37 to 39 days old. They were kept in individual cages, and the greatest care was observed to avoid cage infections. The diet, composed of casein 18 per cent, starch 47 per cent, hydrogenated cottonseed oil 26 per cent, cod liver oil 5 per cent, salt mixture 4 per cent and water, was lacking in vitamin B. The control diet had, in addition, yeast concentrate. Nine rats were fed the deficient diet for three intervals of nineteen, ten and sixteen days, and between these periods of deficient feedings they received a normal diet, i. e., during intervals of one hundred and forty and sixteen days, respectively. At the end of that time, 6 were fed suspensions of 2 cultures of Bact. enteritidis (Gaertner) in 10 cc. of sterile water. Six controls were given almost twice the amount of organisms just mentioned. Examination of the organs of the rats, which were killed from eighteen to forty-eight hours after being fed the suspensions of organisms, failed to disclose Bact. enteritidis in the cardiac blood, liver or kidneys of any of the rats, although the organism was isolated from the intestinal tract of each.

The data, however, throw little light on the natural resistance of the host, since the rats were killed within a day or two after the infection was induced. The capacity for ultimate survival or even the eventual morbidity of the disease was not established. Furthermore, the vitamin B-deficient diet was administered only during short periods. It may be that under such conditions there is little alteration in the permeability of the intestinal wall to bacteria.

However, young rats fed a vitamin B-deficient diet for a period of eight days showed marked depression in their natural resistance to an induced infection with Bact. enteritidis. Ross and Robertson [48] fed cultures of a strain of Bact. enteritidis (Salmonella muriotitis) to these rats, and in 75 per cent of the animals the infection was fatal. When the same quantities were given to normally fed rats, the mortality was 41 per cent.

The natural resistance to infections of the enteritidis group is depressed by depleting animals of their store of vitamin B. The administration of a diet deficient in this vitamin complex to young rats (in which the storage of the vitamin B factors is slight) for a few days may be followed by a decrease in resistance to a subsequently induced infection.

*Bacterium Aertrycke.*—A deficiency of vitamin B in mice may have little effect on a subsequently induced infection with Bact. aertrycke (Lassen [49]). However, Lassen fed 20 white mice a deficient diet [50] for

47. Verder, E.: J. Infect. Dis. **42**:589, 1928.

48. Ross, J. B., and Robertson, E. C.: Am. J. Dis. Child. **43**:547, 1932.

49. Lassen, H. C. A.: Ztschr. f. Immunitätsforsch. u. exper. Therap. **63**:110, 1929.

50. The diet contained casein, rice starch, soya oil, orange juice, salt mixture and cod liver oil. Uninfected mice on this diet survive twenty-three days.

a period of only two days prior to the administration by mouth of 10 drops of an eighteen hour culture of the organism. In 19 mice raised on an adequate diet of white bread and cereals the average duration of life following the administration of the culture was nine and six tenths days. The mice on the deficient diet died in six days. Lassen did not believe the results showed significant differences between the two groups.

In an extended study of the effect of vitamins on infections, Hotta [51] observed no alteration in the natural resistance of mice fed a vitamin B-deficient diet to a subsequent infection with mouse typhoid (Bact. aertrycke). The experiments were thoroughly controlled, and the mortality with varying quantities of the organism from ¼ to 1 minimal lethal dose was observed. The diets were given for periods of from sixteen to eighteen days. Eight animals were tested with each amount, and the experiments were repeated in triplicate. The vitamin B-deficient animals lost considerable weight, but the mortality in this group was no greater than in the normally fed group.

Apparently, the natural resistance of mice is unaffected by depletion of their reserve of vitamin B. This may be due to the fact that certain bacteria are capable of producing vitamin B (Burrows and Jarstadt [52]).

*Clostridium Welchii.*—Dogs fed normal diets and apparently in good health are highly resistant to Cl. welchii. Rose [53] observed marked depression in their natural resistance following a period of feeding on a diet lacking vitamin B.

Positive blood cultures were obtained in the deficient group inoculated with this organism. The results were similar even in those instances in which a minimal quantity of this vitamin complex was given to maintain appetite and body weight. If vitamin B was administered in excess, the blood cultures became negative and continued so for months. Details of the experiments were not published.

### CHRONIC INFECTION: LEPROSY

In an analysis of the geographic distribution of leprosy, Basu [54] observed that this disease is common in India. The diet of the East Indian people consists essentially of rice of low quality, salt in abundance, stale fish or fish especially prepared by being dried a long time in the sun. It is poor in protein and deficient in the vitamin B factors (more prominently in riboflavin). (Pellagra, a disease attributed to deficiency of riboflavin, is, however, seldom seen in India.)

51. Hotta, Y.: Centralbl. f. Bakt. (Abt. 1) **108**:413, 1928.
52. Burrows, M. T., and Jarstadt, L. N.: Am. J. Physiol. **77**:24, 1926.
53. Rose, W. B.: Proc. Soc. Exper. Biol. & Med. **25**:657, 1926.
54. Basu, N. K.: Ztschr. f. Vitaminforsch. **3**:194, 1934.

On the basis of these observations, Basu treated patients with leprosy in an early stage by means of a vitamin B concentrate prepared from yeast. In the course of a month's treatment patients with anesthetic patches showed marked improvement and regained sensation of the skin.

The required duration of the treatment was found to depend on the severity of the disease; the earlier the stage of the leprosy, the quicker the response. Patients with the nodular type showed little improvement.

Similar observations on the distribution of leprosy among populations whose diet is apparently deficient in vitamin B were recently reported by Lamb [55] and by Muir and Henderson.[56]

Muir and Henderson [56] studied the effect of a deficient diet on leprosy in the rat. They inoculated 52 well fed rats subcutaneously and 48 intraperitoneally with rat leproma material. At autopsy, all but 4 showed lesions, and the lymph nodes and spleen and occasionally the liver contained acid-fast organisms. Diets deficient in vitamin A or vitamin B and diets of stale fish and rice were fed to 133 rats. These were inoculated with leproma material subcutaneously or intraperitoneally. All showed lesions at autopsy, but no difference which could be ascribed to the deficient diets was observed in the rate of onset or in the severity of the lesions.

In studying the effect of malnutrition on the pathogenesis of leprosy in the rat by extensive and well controlled experiments Lamb [55] obtained more positive data of a correlation between deficiency of vitamin B and susceptibility to leprosy.

In the rat, leprosy is caused by an organism (Mycobacterium leprae muris) identical morphologically and tinctorially with the bacillus of human leprosy. The rats were inoculated subcutaneously with material from a leprous nodule obtained from a rat and were subjected to many dietary deficiencies.

He observed that a diet of starch foods plus taro root and fish, i. e., a diet moderately deficient in vitamin B and in calcium, repeatedly hastened and extended the development of subcutaneous lesions. Diets moderately deficient in this vitamin complex alone but with a sufficient amount of it to permit suboptimal reproduction produced a similar increase in leprous lesions in the third and fourth generations. In such animals, when intracardiac inoculations of leprous material were made, an extensive increase in visceral leprous lesions, particularly in lesions of the liver, was observed. The spleen, lungs, lymph nodes and skin were infiltrated with organisms and contained microscopic leprous lesions. In the rats fed a normal diet the lesions were limited in size.

55. Lamb, A. R.: Am. J. Hyg. **21**:438, 1935.
56. Muir, E., and Henderson, J. M.: Indian J. M. Research **15**:807, 1928.

These experiments were done on approximately 1,200 rats, and 44 different diets were tested. The results are extremely suggestive, and it may be inferred by analogy that a moderate deficiency of vitamin B in human beings during several generations may reduce the natural resistance of a people to a chronic infection such as leprosy. These observations substantiate the clinical experience that leprosy is endemic in countries where dietary deficiencies have existed for centuries.

### PARASITIC INFECTIONS

*Trypanosoma Equiperdum.*—Rats fed a diet deficient in vitamin B survived an induced infection with T. equiperdum (Reiner and Paton [57]) as long or longer than normally fed animals. Eleven experiments were made on a series of 165 rats. A diet deficient in vitamin B was fed to 108 of the rats and a normal diet to 57, while the number of trypanosomes injected varied in the different experiments. The duration of life showed no statistically significant variation in the two groups, though the trend was in favor of the animals deficient in this vitamin complex. The lack of vitamin $B_1$ seemed more important than that of riboflavin. In starved rats given injections of trypanosomes the authors observed a delay in the appearance of the organisms in the peripheral blood. The rats died of the effects of starvation rather than of the infection. Even in the presence of polyneuritis the infection with T. equiperdum may be milder than in the normally fed animals.

These observations suggest the interesting conclusion that trypanosomes, unlike certain bacteria, do not produce their own vitamin B, nor are they capable of surviving as well in an environment completely depleted of this vitamin complex as in one adequate in this respect. Studies on infections induced by protozoa must take the cultural needs of the protozoa into consideration. I [58] observed this to be true in the case of copper. Though an excess of copper in the diet of rats raised their resistance to Trypanosoma lewisi, depletion of copper in the diet inhibited the active growth of trypanosomes in the peripheral blood.

The experiments of Reiner and Paton [57] therefore suggest that trypanosomes may need an adequate supply of vitamin $B_1$ concentrate for growth and shed little light on the question of the importance of vitamin B in the maintenance of the natural resistance of rats to infection.

*Ascaridia Perspicillum.*—Chicks fed a diet deficient in vitamin B appeared less resistant to the parasitism of round worms than normally fed chicks (Zimmerman, Naormi, Vincent and Ackert [59]).

57. Reiner, L., and Paton, J. B.: Proc. Soc. Exper. Biol. & Med. **30**:345, 1932.
58. Perla, David: Am. J. Hyg. **19**:514, 1934.
59. Zimmerman, N. B.; Naormi, B.; Vincent, L. B., and Ackert, J. E.: J. Parasitol. **12**:164, 1926.

A number of young chicks were divided into three groups. One group was given an adequate normal diet; the second, a synthetic complete diet, and the third, a synthetic diet deficient in vitamin B. The groups were maintained on their diets during a period of two weeks and then parasitized with round worms. This was done by feeding the chicks approximately equal numbers of embryonated eggs of A. perspicillum. Three weeks later, the chicks were killed and the number and size of the worms in the intestines determined.

The worms in the group fed the diet deficient in the vitamin complex were more numerous and grew to be significantly longer than those in chicks of the same age raised under the same conditions but given adequate rations.

*Ascaridia Lineata.*—Similar studies were more extensively carried out some years later by Ackert and Nolf,[60] using A. lineata as the infecting nematode. Three experiments were made, involving 135 chickens, to ascertain whether vitamin B is a factor in the natural resistance of chickens to this intestinal round worm. The source of the vitamin complex was baker's yeast.

All the chickens were pure bred white leghorns and were secured as 1 day old chicks. They were reared in screened parasite-free animal houses under sanitary conditions and were maintained on an adequate diet. When the chicks were 49 days old they were divided in such a manner that there were three groups available for each of the experiments. One group was fed a control natural diet made up of the following foods in the percentages given: corn chop, 60; wheat, 20; oats, 10; meat meal, 22; hydrogenated cottonseed oil, 2; starch, 9; cod liver oil, 2, and salt mixture, 5. The second group received a diet containing the following foods in the percentages given: casein (free from vitamin B), 20; polished rice, 52; cod liver oil, 5; hydrogenated cottonseed oil, 10; salt mixture, 4, and bakers' yeast, 6. The third group received the same diet without the yeast. After the chicks had been maintained on these diets for two weeks, each bird was fed 500 embryonated eggs of A. lineata. In one experiment, only 50 embryonated eggs were given each bird.

Forty-five chicks were used in each of the three experiments. Three weeks after they had become infested, all were killed, the intestinal contents were removed, and the worms were measured by magnification. Since the parasites are usually eliminated during the first month, the numbers remaining in the intestines after three weeks were significant. The length of the worms as an index of the degree of growth was important, since the worms did not grow to be as great a length in old, more resistant chickens as in young chickens.

The results were similar in the three experiments. The chickens deficient in vitamin B had more worms than similar groups which had had adequate amounts of this vitamin complex in their rations. The larger number of worms was attributed in part to partial paralysis of the intestine due to lack of vitamin B, the weakened peristalsis probably aiding the worms to remain in their habitat.

60. Ackert, J. E., and Nolf, L. O.: Am. J. Hyg. **13**:337, 1931.

It is interesting that larger worms were found in chickens fed the diet containing yeast than in those given a diet without yeast. It is suggested that yeast may contain a factor favorable to the growth of the worms. Nevertheless, it was clear that deficiency of vitamin B favors the multiplication and retention of the nematodes in chickens.

*Ancylostoma Canium.*—Nagoya [61] studied the effect of deficiency of vitamin B on the natural resistance of puppies and of white mice to A. canium.

The puppies used were from 2 to 3 months of age. Five were fed a diet consisting of casein, polished rice, cod liver oil, salt mixture and Japanese radish juice. Five received, in addition, yeast as a source of vitamin B, and 5 were deprived of vitamin A. At the end of two weeks, all were infected by introducing 10,000 larvae into the stomach by catheter. From twenty-four to seventy-two hours later, all were killed. Serial sections were made of the lungs, liver, stomach and intestines, and an effort was made to count microscopically the actual number of larvae per given unit of tissue. The total number of larvae was then estimated. Greater numbers of organisms were found in the lung, liver, stomach and intestines in the vitamin B-deficient puppies than in the normal ones. Similar results were obtained if the larvae were administered percutaneously by placing them on the shaved skin and keeping them close to the body by means of a gauze bandage.

White mice, an abnormal host for A. canium, when fed a diet deficient in vitamin B also showed within a period of from twenty-four to seventy-two hours after percutaneous infection or oral infection greater numbers of larvae than mice normally fed (Nagoya [62]).

Deficiency of vitamin B in puppies and white mice lowers their natural resistance to A. canium.

### VIRUS DISEASES

In view of the importance of vitamin $B_1$ in the processes of oxidation and of carbohydrate metabolism in the nervous system one might anticipate some influence of a deficiency of this factor on the susceptibility of animals to viruses. Few experimental studies on this subject are available. (See part VIII, on vitamin B and the virus of yellow fever.)

The use of water-soluble vitamin $B_1$ in the treatment of herpetic stomatitis and herpes labialis was recommended by Gerstenberger,[63] who obtained apparently beneficial results. He believed susceptibility to these conditions was increased by relative lack of vitamin $B_1$ in the diet.

Cowdry, Lucas and Neff [64] attempted to determine whether deficiency of vitamin $B_1$ or of vitamin G (or $B_2$) increases the susceptibility of rats to intracerebrally injected herpetic virus.

61. Nagoya, T.: Jap. J. Exper. Med. **9**:587, 1931.
62. Nagoya,[61] p. 603.
63. Gerstenberger, H. J.: Am. J. Dis. Child. **26**:309, 1923.
64. Cowdry, E. V.; Lucas, A. M., and Neff, C. F.: J. Infect. Dis. **57**:174, 1935.

The rats used were 4 weeks old when placed on the diets and were maintained on them for a period of from twenty-three to thirty days prior to the intracerebral injection of a strain of herpetic virus. The virus was obtained from infected rabbits by diluting emulsions of brain to from 1:10 to 1:1,000,000. The results were observed over a period of seventy-four days.

The deficient basal diet used consisted of the following foods in the percentages given: casein, 18; fat, 14; cod liver oil, 2; Osborne and Mendel salt mixture, 4, and dextrinized cornstarch, 62. The $B_1$-deficient diet was made by adding 8 Gm. of autoclaved yeast to each 100 Gm. of the basal diet. The $B_2$-deficient diet was made by adding concentrated alcoholic extract of wheat in amounts equivalent to 50 Gm. of whole wheat for each 100 Gm. of deficient diet.

The results indicated that rats deficient in vitamin $B_1$ or in vitamin $B_2$ were as resistant as their controls to intracerebrally inoculated herpetic virus. The differences in longevity with larger amounts of the virus were not significant. Rats deficient in either of these vitamins showed a slightly greater number of deaths after intracerebral injection of the virus, but the difference is not sufficient for one to conclude that the deficiencies reduced the resistance of the rats to herpes.

## SUMMARY

The evidence on the effect of deficiencies in the vitamin B factors on natural resistance to induced infections in experimental animals is difficult to analyze. A number of papers reporting a marked decrease of resistance in pigeons fed a diet exclusively of polished rice cannot be considered in this regard since other deficiencies in diet were present, the importance of which was not controlled. It is true that such pigeons suffer manifestly from beriberi, a disease due to deficiency of vitamin B, but unrecognized effects on cellular metabolism must be created by the complete avitaminosis, the imbalance in the diet and the element of starvation. Some of this work suggests that the drop in resistance in the bird with beriberi is due to a drop in the body temperature, since this condition alone, induced by a single large dose of an antipyretic, aminopyrine, is also associated with a similar drop in resistance. It seems that this may be a coincidence, for certainly other effects are produced by a deficiency of vitamin B on the one hand and, on the other hand, aminopyrine may be injurious to pigeons when given in large doses.

Nevertheless, pigeons fed a diet of polished rice show significant depression in resistance to infectious agents against which they normally possess high natural resistance. In such deficient birds infections are readily induced by injecting cultures of Pneumococcus, N. meningitides, Bact. coli, Bact. enteritidis and Erys. rhusiopathiae (swine erysipelas) and prove fatal.

A few studies have been reported on pigeons with a dietary apparently deficient only in vitamin B. In such birds the natural resistance

is altered. Pneumococcus and B. anthracis, nonpathogenic to normally fed pigeons, become pathogenic in those fed a diet deficient in vitamin B, inducing severe and often fatal disease. Infection with A. perspicillum and with A. lineata is also definitely more severe in vitamin B-depleted pigeons than in those normally fed.

In rats, induced infection with Bact. enteritidis is definitely much more severe if the animals have previously been fed a diet deficient in vitamin B. Such rats are also more susceptible to the effect of diphtheria toxin, though the pattern of response is unaltered.

Leprosy occurs endemically in human beings in those geographic areas and countries where the diet of the population is particularly deficient in vitamin B. Experimentally, rats fed a diet containing insufficient amounts of this complex but adequate to maintain suboptimal growth and reproduction show marked depression in natural resistance to leprosy in the third and fourth generation.

On the other hand, deficiency of vitamin B does not significantly shorten the life span of rats infected with T. equiperdum. This is, however, not so significant, since the infection is extremely virulent in the normally fed animals, and protozoa of this type apparently require vitamin B for their own nutrition and growth.

Mice, though susceptible to the effects of depletion in vitamin B, do not demonstrate increased susceptibility to Bact. aertrycke, the etiologic organism of mouse typhoid. This may be due to the fact that certain bacteria, particularly those infecting the gastrointestinal tract, are capable of producing vitamin B.

Dogs fed a diet deficient in vitamin B become susceptible to infection with Cl. welchii, to which they normally show high resistance. The natural resistance of vitamin B-deficient puppies and mice is definitely decreased to induced infection with A. canium.

In summary, deficiency of vitamin B in pigeons, dogs and rats lowers their natural resistance to subsequently induced bacterial and nematode infection. Mice suffering from deficiency of vitamin B, though more susceptible to induced hookworm infestation than normally fed mice, show little alteration in response to mouse typhoid, and rats demonstrate no significant drop in resistance to infection with T. equiperdum under a similar dietary régime. Though the explanation of these apparent inconsistencies is not entirely clear at present, it is evident in spite of them that vitamin B is an essential element in the maintenance of natural resistance to infection in birds and many mammals.

## VII. EFFECT OF DEFICIENCY OF VITAMIN B ON NATURAL RESISTANCE TO POISONS AND TOXINS

*Diphtheria Toxin.*—Rats deficient in vitamin B succumb to smaller amounts of diphtheria toxin than normal animals fed a complete diet (Werkman, Baldwin and Nelson [65]). The ability of the rat to produce antitoxin is undisturbed. The normal rat produces as little as from 0.5 to 1 unit per cubic centimeter after five injections of toxin in sublethal amounts. The absorption of diphtheria toxin by the cells of the vitamin B-deficient rats is not greater than that by the cells of normally fed animals. The toxin is found in the serum, unaltered, for hours after its injection. It produces a progressive drop in blood pressure on the second or third day after the injection, which is more pronounced and rapid in the deficient rats.

*Clostridium Welchii Toxin.*—It was emphasized by Rose, Rose and Kolmer [66] that in testing the effect of a deficiency in vitamin B on the resistance of animals to toxin it is important to evaluate the degree of starvation which characterizes this deficiency. Since it is well known that partial anorexia develops in animals subsisting on a diet deficient in vitamin B and, as a result, loss in weight, these authors fed their control rats a partial starvation diet simply by limiting the intake of the basic diet. They determined the resistance of these animals to the toxin of Cl. welchii, together with that of young rats fed a diet deficient in vitamin B. Thirteen partially starved animals and an equal number of deficient animals were tested. The results obtained showed no significant differences in resistance between the controls and the deficient animals. Apparently, both the animals that were partially starved and the animals deficient in vitamin B showed some depression in resistance as compared with normally fed animals. In the opinion of the investigators the results indicate that resistance to the toxin of Cl. welchii varies with the degree of nutritive disturbance incident to the deficiency disease and that it does not appear to depend on any specific effect of this vitamin complex; that is, when the caloric intake of the controls was maintained at a level corresponding to that of the vitamin-deficient animals, the controls became as susceptible to the toxin of Cl. welchii as the rats deficient in vitamin B.

While it is true that partial starvation in itself lowers resistance, it does not follow from the experiments of Rose, Rose and Kolmer [66] that deficiency of vitamin B may not be associated with depression in resistance apart from the factor of inanition. Further, the investigators stated that both the starved and the vitamin-deficient animals had gross

65. Werkman, C. H.; Baldwin, F. M., and Nelson, V. E.: J. Infect. Dis. **35**:549, 1924.

66. Rose, S. B.; Rose, W. B., and Kolmer, J. A.: J. Infect. Dis. **59**:50, 1936.

hematuria. As has been pointed out by Willis, deficiency of vitamins in the diet leads to a flare-up of the anemia associated with an infection with Bartonella muris. It is possible that in the experiments reported by Rose, Rose and Kolmer [66] the anemia associated with this infection may have developed. This would account for the hematuria and, of course, would complicate and invalidate their results. No mention is made of the blood picture of these animals.

*Drugs.*—Pigeons fed a diet of polished rice are not more sensitive than normally fed pigeons to the action of epinephrine, atropine, choline or histamine, nor is there any difference in the type of response to any of these drugs (von Leeuwen and Verzár [67]).

67. von Leeuwen, W. S., and Verzár, F.: Ber. ü. d. ges. Physiol. **7**:422, 1921.

*(To Be Concluded)*

# Notes and News

**University News, Promotions, Resignations, Appointments, Deaths, etc.**—Ernest W. Goodpasture, professor of pathology at Vanderbilt University, has been awarded the research medal of the Southern Medical Association in recognition of his study of virus diseases.

Ralph L. Ferguson, formerly of the department of pathology, Ohio State University, has been appointed associate professor of bacteriology at Loyola University, Chicago.

**Society News.**—Officers of the Pathological Society of Philadelphia for 1938 are: Baxter L. Crawford, president; Jefferson H. Clark, vice president, and Herbert L. Ratcliffe, secretary-treasurer.

The fifty-third meeting of the Association of American Physicians will be held at the Chalfonte-Haddon Hall Hotel, Atlantic City, N. J., May 3, 4 and 5, 1938, in conjunction with the congress of American Physicians and Surgeons.

At its recent meeting in Washington, the Society of American Bacteriologists bestowed the Eli Lilly Award of $1,000 on Frank L. Horsfall, of the Rockefeller Institute for Medical Research, as the investigator under 31 years of age who accomplished the most important work in bacteriology or immunology in the United States during the preceding year. One result of Dr. Horsfall's work has been the development in rabbits of serum protective against pneumonia.

At its recent meeting in Washington, the Society of American Bacteriologists elected Paul F. Clark president, Arthur T. Henrici vice president and Ira L. Baldwin secretary-treasurer.

The annual meeting of the American Association of Pathologists and Bacteriologists will be held in conjunction with the congress of the Association of American Physicians at Chalfonte-Haddon Hall, Atlantic City, N. J., May 3 and 4, 1938.

The annual meeting of the American Association of Immunologists will be held in Atlantic City, May 2 and 3, 1938.

# Abstracts from Current Literature

To Save Space the Original Titles of Abstracted Articles Sometimes Are Shortened

## Experimental Pathology and Pathologic Physiology

Calcium Deposits in Nerve Cells of the White Rat After Injection of Urea and Cholesterol. R. C. MacCardle, Anat. Rec. **67**:81, 1936.

Daily intraperitoneal injection of 4 cc. of olive oil saturated with cholesterol and of 5 cc. of a 3 per cent aqueous solution of urea induced deposition of calcium in ganglion cells of the medulla and in motor cells of the spinal cord of the white rat. The deposition of calcium was generally preceded by accumulations of fat. No calcium soaps could be demonstrated. The precipitation of calcium is believed to be favored by the increase in alkalinity produced by urea.

R. J. Lebowich.

Effects of Gonadotropic Hormones on Experimental Tuberculosis. M. M. Steinbach and S. J. Klein, J. Exper. Med. **65**:205, 1937.

Experimental tuberculosis in rabbits and guinea-pigs was favorably influenced by administration of the gonadotropic substance from the urine of pregnant women and the serum of pregnant mares. No retardation of the disease was obtained by the use of an extract of the anterior lobe of the pituitary or of emmenin (Collip's placental extract). The results suggest that the gonadotropic substance mentioned may be a factor in the temporary amelioration of symptoms observed in tuberculous women during pregnancy.

From the Authors' Summary.

Tissue Reaction After Experimental Administration of Colloidal Silicic Acid. G. F. Koppenhöfer, Virchows Arch. f. path. Anat. **297**:271, 1936.

To what extent the tissue reaction in silicosis is the result of mechanical or of physicochemical action is still a matter of question. In the experiments reported here the material used was a 0.25 per cent colloidal silicic acid sol which contains no free electrolyte. It was injected daily intravenously into rabbits in doses of 1 cc. and, later of 2 cc., representing, respectively, 2.5 and 5 mg. of silicic acid. The animals were killed after varying intervals and their tissues examined histologically. Diffuse fibrosis was observed, which was most marked in the liver but was present also in other organs, especially the kidney, spleen, bone marrow and lymph nodes. This "fibroplastic" reaction the author considers the characteristic and essential effect of the action of colloidal silicic acid, on which the harmful action of silicon compounds depends. The reaction is due to the negatively charged particles of the silicic acid sol. This action may be diminished or neutralized by positively charged sols, such as aluminum hydroxide. This accounts in part for the variable tissue reaction to different kinds of silicon compounds. The difference between the diffuse tissue reaction of experimental and spontaneous asbestosis and the nodular fibrosis that develops in the presence of quartz and stone dust has a mechanical basis. The asbestos needles, because of their shape, are held more or less widely separated in the tissues. Liberation of colloidal silicic acid from them results in diffuse fibrosis. Quartz and stone particles are transported by the lymphatic channels. They are held and agglomerated here and in the lymph nodes. Liberation of silicic acid leads to a more localized, i. e., nodular reaction about the masses of foreign particles. Because the silicic acid is in greater concentration, the fibrotic nodular reaction is more intense and progresses to hyalinization and later to necrosis through loss of blood and lymph supply.

O. T. Schultz.

## Pathologic Anatomy

DISSECTING ANEURYSM OF THE AORTA. R. E. GLENDY, B. CASTLEMAN and P. D. WHITE, Am. Heart J. **13**:129, 1937.

An analysis has been made of 13 cases of dissecting aortic aneurysm directly related to the death of the patient, and of 6 other cases found among 8,200 necropsies on persons of all ages at the Massachusetts General Hospital. Included is the report of a case in which dissection occurred between the coats of the wall of a coronary vessel. Among the 13 cases of acute aneurysm the diagnosis was made correctly during life in 2, bringing the total of correct antemortem diagnoses recorded to 13 or more. Survival after the onset of symptoms among these 13 cases averaged approximately four days, with 3 exceptions, in which the duration of life was six, eight and fifteen weeks, respectively. Among the incidental cases there was 1 in which autopsy showed a "double-barreled" aorta, which condition was unrelated to the patient's death. Anatomically the predominant features are (1) rupture of the intima near the aortic valve ring, (2) dissection of the medial coat of the aorta and (3) some degree of medial degeneration. Medionecrosis aortae idiopathica cystica (Erdheim) was present in 6 of the acute cases and in 2 of the six incidental cases. Syphilis was not an etiologic factor in any case. Proximal dissection involving the mouths of the coronary vessels occurs at times and may cause confusion in differentiating dissecting aneurysm and coronary thrombosis. Interest in this dramatic condition is mounting as shown by the increasing number of reports of cases in recent years in which the condition was correctly diagnosed.

FROM THE AUTHORS' SUMMARY.

EXTREME CARDIAC ENLARGEMENT. J. S. GOLDEN and W. A. BRAMS, Am. Heart J. **13**:207, 1937.

Although only thirty-eight reports of hearts weighing 1,000 Gm. or more could be found in the literature of the past century, nine such specimens were observed from patients admitted to the Cook County Hospital in the past eight years. The unreliability of statistics based on small numbers of patients is again emphasized when the data on this group of nine hearts weighing 1,000 Gm. or more are compared with the reports in the literature. Only one of these nine large hearts showed pericardial adhesions, while in almost half of those previously reported the large size was supposedly due to pericardial adhesions, either alone or in combination with valvular disease. The great predominance of males in both series of patients is striking and cannot be easily explained. Deformity of the aortic valve, usually insufficiency, was the most frequent valvular lesion found in nine patients and in those previously reported. A patient whose heart weighed 1,475 Gm. is reported on in detail, and the point is emphasized that no adequate explanation can be offered either from clinical or anatomic study of this case. The term "idiopathic" is probably appropriate in this instance, as the cause is not known and the anatomic changes are far from adequate to explain such extreme enlargement and hypertrophy.

FROM THE AUTHORS' SUMMARY.

BLASTOMYCOSIS OF THE HEART. R. D. BAKER and E. W. BRIAN, Am. J. Path. **13**:139, 1937.

Blastomycosis of the heart was encountered at autopsy in two cases of generalized infection with Blastomyces dermatitidis. In each case autopsy showed diffuse pericardial blastomycosis, a large blastomycotic tubercle of the right atrial wall and involvement of the corresponding endocardium. From the latter site, in both cases, organisms apparently entered the blood stream to produce miliary pulmonary blastomycosis. Evidences of cardiac insufficiency, dependent probably on the cardiac blastomycosis, were present in both. Blastomycosis of the heart may also

develop as part of generalized miliary blastomycosis and possibly by retrograde lymphatic extension from infection in mediastinal nodes. Blastomycosis is similar to tuberculosis in respect to cardiac involvement.

FROM THE AUTHORS' SUMMARY.

THE HISTOPATHOLOGY OF CANINE DISTEMPER. W. A. DE MONBREUN, Am. J. Path. **13**:187, 1937.

The most important literature on canine distemper is reviewed. In a histologic and cytologic study of both the natural and the experimental disease in puppies, lesions that the author believes to be characteristic of the disease are described. The virus of canine distemper has a definite affinity for vascular endothelium and for cells of the reticulo-endothelial system. The virus spreads in the body of the host mainly by way of the blood stream. No evidence has been obtained that it passes along the nerve pathways. The natural route of infection is by way of the respiratory tract. The occurrence of nuclear inclusions, heretofore unreported, in liver cells, bronchial epithelial cells, glandular cells of the stomach and intestine and bile duct epithelial cells, as well as of cytoplasmic inclusions in the bile duct epithelial cells, is described. A heretofore unreported clinical type of the disease with characteristic microscopic changes is described and has been reproduced in puppies. Various clinical types of the disease have been induced in puppies with a single strain of virus. In histologic and cytologic aspects the disease in dogs is quite similar to that in ferrets.

FROM THE AUTHOR'S SUMMARY.

BASOPHIL INFILTRATION IN THE NEUROHYPOPHYSIS. D. C. LEARY and H. M. ZIMMERMAN, Am. J. Path. **13**:213, 1937.

One hundred fifty-three pituitaries have been studied in serial sections. Both sexes and all age groups were represented, as well as sixty-seven hypertensive and eighty-six nonhypertensive persons. Basophil infiltration of very slight to advanced degree was seen in the posterior lobe in 64.7 per cent of the cases, including the hypertensive and nonhypertensive groups as well as all age groups. Significant basophil infiltration was found in fifty-two of sixty-seven hypertensive and in twenty-two of eighty-six nonhypertensive persons. Increased basophil infiltration was seen in twenty-nine hypertensive persons (nineteen males and ten females) and in six nonhypertensive persons (all males). The only patients under 20 years to show any degree of infiltration at all were a child of 6 with hypertension of unknown cause and a white girl of 17 who died of bromide poisoning. Increased infiltration was seen in only two persons under the age of 40. It is concluded that significant basophil infiltration is much more common in hypertensive than in nonhypertensive persons, more common in males than in females, and more common after the fortieth year. Confirmation of the observation of Kraus and Traube that severe basophil infiltration is more common in the hypersthenic person could not be demonstrated. Adenoma of the pituitary was seen in ten hypertensive persons and in one nonhypertensive person. The types were as follows: chromophobe in six, eosinophil in one, basophil in two and mixed basophil and chromophobe in two.

FROM THE AUTHORS' SUMMARY.

## Microbiology and Parasitology

STUDIES ON THE YELLOW FEVER VIRUS. M. THEILER and H. H. SMITH, J. Exper. Med. **65**:767, 787 and 801, 1937.

In vitro cultivation of the virus of yellow fever resulted in a change in pathogenicity, this change varying with the medium on which it was cultivated. The mediums employed consisted of whole mouse embryo, chick embryo and the testicular tissues of mice and guinea pigs. Though the virus which was grown on mouse embryo did not, after subcutaneous injection, produce fatal infection in

monkeys and hedgehogs, it gave rise to a generalized infection of the blood. The viscerotropic virulence was diminished, but not the neurotropic virulence. Grown on chick embryo, the virus lost viscerotropic virulence; injected intracerebrally, it caused only a mild febrile reaction. Cultivated on testicular tissue, it produced death from encephalitis in monkeys inoculated intracerebrally.

Vaccination of monkeys by subcutaneous inoculation led to immunity of a high grade, unrelated to the amount of virus inoculated. Vaccination of eight normal persons with the virus resulted in minimal reactions, and yellow fever antibodies were demonstrable in from three to four weeks after inoculation. Unmodified strains of the virus grew well in a medium consisting of minced mouse embryo brain tissue and Tyrode solution containing 10 per cent normal monkey serum. Continued cultivation in this medium was found to be ideal for promoting subsequent growth on whole mouse tissue medium, on which the neurotropic properties of the virus became increased. In vitro cultivation of the virus on monkey tissues failed.

FREDERICK STENN.

INFECTIOUS CATARRH OF MICE. J. B. NELSON, J. Exper. Med. **65**:833, 843 and 851, 1937.

A natural outbreak of infectious catarrh in a colony of Swiss mice is reported. The disease was generally characterized by a peculiar chattering during life and by rhinitis, otitis media and pneumonia at autopsy. The pneumonia was slowly progressive and terminated fatally in a high percentage of cases. The mortality in a group of seventy-five naturally infected mice over a period of eleven months was 95 per cent. The disease was readily reproducible in susceptible mice by nasal instillation of exudate from any locus of infection. It was also transmissible by direct contact. In both naturally and experimentally infected animals there was an incubation period of ten days or more before symptoms were apparent. Recovery from the disease was not observed.

Small gram-negative cells resembling the so-called coccobacilliform bodies of fowl coryza were regularly found in the nasal and middle ear exudate of mice naturally and experimentally infected with catarrh. These bodies were successfully isolated from exudates and cultivated in tissue cultures. There was no microscopic evidence, however, of their multiplication in an ordinary nutrient medium enriched with blood. They could be filtered through collodion membranes with an average pore size of 640 millimicrons and hence were separable from secondary bacteria. The size of the bodies in stained films averaged between 0.3 and 0.4 micron. A second organism, cultivable in fluid blood medium with the formation of compact clumps and similar to the X bacillus of chickens, was also isolated from infected mice.

Evidence is presented that the etiology of infectious murine catarrh is specifically referable to the coccobacilliform bodies. The disease was regularly produced in normal mice by nasal instillation of primary tissue cultures. In the presence of the X bacillus, transfers of primary cultures were usually uninfective. Pure cultures, however, retained their pathogenicity through as many as twelve transfers. The onset and progress of the experimental disease were somewhat retarded in comparison with the natural disease, but in general there was a close parallel. Mice given injections of the cultures did, however, show a significant decrease in the incidence of rhinitis. Transmission by direct contact was demonstrated in the presence of rhinitis but not in its absence. FROM AUTHOR'S SUMMARIES.

THE AUTOLYTIC SYSTEM OF PNEUMOCOCCI. R. J. DUBOS, J. Exper. Med. **65**:873, 1937.

Living pneumococci contain a group of enzymes, the bacteriolytic system, capable of causing lysis of heat-killed pneumococci (R and S variants, irrespective of the type from which they may be derived). This lysis is expressed by loss of

the Gram staining reaction, disintegration of the cell body and clearing of the bacterial suspension. Under certain conditions of treatment with the bacteriolytic complex, it is possible to render the cocci gram-negative without changing their morphologic character or causing any appreciable clearing of the cell suspension. The enzyme responsible for this change has been partially purified, and some of its properties have been described. The cellular structure which is responsible for the gram-positive reaction of pneumococci is resistant to proteolytic enzymes and is still present when tryptic digestion has reduced the heat-killed cell to a body which has lost 75 per cent of its original weight and contains only 8 per cent nitrogen. The same enzyme preparation which attacks pneumococci is also capable of liberating reducing sugars from some acetylaminodextrose glycuronides of animal and bacterial origin. Dubos considers and discusses the possibility that one and the same enzyme in the autolytic complex is capable of attacking both types of substrates.

FROM AUTHOR'S SUMMARY.

PROPAGATION OF RABIES VIRUS IN TISSUE CULTURE. L. T. WEBSTER and A. D. CLOW, J. Exper. Med. **66**:125, 1937.

Rabies virus was propagated in a medium of serum and Tyrode solution containing either embryo mouse brain or embryo chick brain. The culture virus reached a titer of $3 \times 10^{-5}$ cc. after four days' incubation at 37 C. and survived at least two months at 5 C. in the liquid or dry state.

FROM AUTHORS' SUMMARY.

COMPARISON OF MENINGEAL AND OTHER STRAINS OF HAEMOPHILUS INFLUENZAE. D. A. W. WEISS, J. Infect. Dis. **60**:213, 1937.

Morphologic characteristics did not serve to differentiate meningeal and respiratory strains of Haemophilus influenzae. Organisms freshly isolated from the blood of patients with influenzal meningitis were invariably short, while the smears from the spinal fluid showed many long threadlike forms. The strains from blood and also those from spinal fluid invariably gave smooth colonies on Levinthal agar. Smears from these colonies revealed the short coccobacillary form, although pleomorphism was occasionally observed.

Forty-three meningeal strains were tested for production of indol. With only five of the strains was the test negative. However, of the nineteen respiratory strains, ten formed indol, and hence this test can be of little value in differentiating between the two. One hundred consecutive spinal fluids obtained from patients with miscellaneous diseases were tested for the presence of indol, and in five of these the test was positive; three were from patients with influenzal meningitis and two were from patients with cyst of the brain and mastoiditis, respectively.

A number of stock strains of H. influenzae were classified by fermentation reactions and by serologic tests. A number of meningeal strains that were found to be similar in the fermentation tests seemed to be closely related antigenically in agglutination tests.

FROM AUTHOR'S SUMMARY.

BACTERIOLOGIC STUDY OF DYSENTERY IN INFANTS AND CHILDREN. E. HAYNES, J. Infect. Dis. **60**:251, 1937.

A study of dysentery in forty infants and children is reported. The Flexner bacillus was obtained from thirteen of the patients, including two from whom this organism was obtained in blood culture. It seems that the dysentery bacillus can enter the blood stream, but the frequency or time of its appearance there is not yet known.

The Sonne dysentery bacillus was isolated from five of the patients; a bacillus obtained from a sixth, which unfortunately was not completely studied, probably was a Sonne bacillus. Sonne dysentery is possibly endemic in this country, but because

the disease is usually mild and because of the difficulty in recognizing the organism, cases are not frequently reported. It seems probable that adult carriers of this organism may be quite common and that some of the mild food infections may be caused by it.

Unless a titer of well over 1:100 is obtained, agglutination is of doubtful value for diagnosis, as agglutinins may be present in lower titers in the blood for some time after the disease, and negative agglutination tests do not exclude the disease.

FROM AUTHOR'S SUMMARY.

BRUCELLA INFECTION IN WHITE MICE. C. H. SINGER-BROOKS, J. Infect. Dis. **60**:265, 1937.

Normal white mice succumb quickly to large doses of living cultures of Brucella (from 300,000,000 to 400,000,000 organisms).

Normal mice subjected to smaller doses (from 30,000,000 to 300,000 organisms) manifest no symptoms of infection but show necrotic areas in the liver and enlargement of the spleen. Infections of from 100,000 to 1,000 organisms do not produce gross anatomic changes, but the organisms are harbored in various tissues for long periods of time.

Protection against fatal infection was conferred by immunization with heat-killed vaccine in 95 per cent of the animals treated.

Pregnancies in immunized mice subjected to lethal infection are not terminated by abortions. The young survive and develop into healthy adults.

Immunized mice subjected to small infections do not manifest any appreciable increase in ability to free their tissues from the organisms as compared with control mice.

FROM AUTHOR'S SUMMARY.

FURTHER STUDIES ON BACTERIUM NECROPHORUM ISOLATED FROM CHRONIC ULCERATIVE COLITIS. G. M. DACK, L. R. DRAGSTEDT and T. E. HEINZ, J. Infect. Dis. **60**:335, 1937.

Bacterium necrophorum has been commonly found associated with ulceration of the colon in man and monkeys. In chronic ulcerative colitis in man it has been found in enormous numbers in the lesions cultured at proctoscopic examination. It has outnumbered other organisms in the severely diseased isolated colons of patients on whom ileostomies have been performed. It has not been recovered from the bowel after healing. It appears to be present in the bowel, since it readily appears when necrotic lesions are present in the colon, regardless of the inciting cause, i. e., bacillary dysentery, trauma, etc.

Bacterium necrophorum does not appear to be a clearly defined bacterial species and has received many different names. It is similar to, if not identical with, what is called in the literature Bacillus funduliformis.

FROM AUTHORS' SUMMARY.

BOVINE MASTITIS IN RELATION TO MILK-BORNE EPIDEMICS. D. J. DAVIS, J. Infect. Dis. **60**:374, 1937.

Veterinarians emphasize the increasing frequency of mastitis in cows. A corresponding increase is noted in milk-borne epidemics of disease traced to infected udders. This parallelism indicates the importance of intensive studies of the problem of mastitis in all its relations.

The udder is not infrequently a reservoir of certain bacteria, especially streptococci and staphylococci, dangerous to man. The possible sources of such organisms have been studied. The data presented relate to human sources of streptococci and staphylococci and to animal sources of streptococci, especially the tonsils of cows and hogs.

Experiments indicate clearly that hemolytic streptococci applied to the teat surface will readily ascend the teat ducts to the milk cisterns, setting up an infec-

tion. Similar experiments with strains of staphylococci have given negative results. However, when staphylococci of human origin are injected into the lactiferous duct a short distance above the meatus infection occurs, resulting in easily recognized mastitis. The possible role of injuries in both streptococcic and staphylococcic infections is emphasized.

A brief presentation of the anatomy and histology of the teat ducts pertinent to this problem is given; also some observations relating to the pathology of the udder as a focus of disease.

FROM AUTHOR'S SUMMARY.

ROLE OF THE GARBAGE-FED HOG IN THE PRODUCTION OF HUMAN TRICHINOSIS. M. C. HALL, Pub. Health Rep. **52**:873, 1937.

Garbage-fed swine harbor trichinas between three and five times as frequently as do grain-fed swine, and hence are specially important as sources of human trichinosis.

Trichinosis in swine is apparently traceable to the eating of uncooked pork scraps in garbage, table scraps, swill and similar things, much more often than it is traceable to the eating of rats by swine.

The garbage-feeding industry, as ordinarily carried on, is dangerous to the health of man and livestock, esthetically objectionable and often economically unsound.

Suggestions are made for the elimination of the dangers and nuisances associated with the garbage-feeding industry and with feeding of table scraps and similar things on the farm. Cooperation between scientists, practicing physicians, engineers, packers and the swine industry is recommended as the best attack on the problem.

FROM AUTHOR'S SUMMARY.

SOME CYTOLOGIC FEATURES OF VACCINIAL KERATITIS IN THE RABBIT. A. J. RHODES and C. E. VAN ROOYEN, J. Path. & Bact. **44**:357, 1937.

Some cytologic features of vaccinial keratitis in the rabbit are described in detail as observed from the first to the thirteenth day following vaccination. The presence of acidophilic inclusion bodies within fibroblasts is described. These appear to be a specific feature of vaccinial keratitis, not having been observed in keratitis produced by bacterial, chemical or mechanical means. The significance of these structures has been investigated by appropriate methods, and arguments in favor of their being aggregates of elementary bodies, which constitute the virus itself, are adduced. It has been demonstrated that both Guarnieri bodies and the fibroblastic inclusions fail to develop in the cornea of rabbits which have been previously actively immunized against vaccinia virus.

FROM AUTHORS' SUMMARY.

TRACHOMA. A. CUÉNOD and R. NATAF, Arch. Inst. Pasteur de Tunis **26**:1, 1937.

Lice were successfully inoculated with trachomatous material, with transfer in series. Rickettsia-like bodies were demonstrated both in and around the epithelial cells of the lower intestines. The virus appeared to multiply in the testicles of guinea pigs without immediate apparent damage to the tissues, which showed microscopically punctiform cellular inclusions identical with those noted in trachomatous pulp. Transfers from guinea pigs to lice were successful. Rabbits, a rat, Macacus inuus and a man were also infected. The human inoculation indicated that trachomatous follicles of the conjunctiva may be produced by the inoculation of a culture of rickettsia obtained by the inoculation of lice with trachomatous pulp. In the tears and in desquamated epithelial cells were noted rickettsia-like forms unlike those of the louse but identical with those described by Halberstädter and Prowazek under the name of inclusions of Chlamydozoa.

M. S. MARSHALL.

## Immunology

THE PROLONGED COAGULATION TIME SUBSEQUENT TO ANAPHYLACTIC SHOCK. H. EAGLE, C. H. JOHNSTON and I. S. RAVDIN, Bull. Johns Hopkins Hosp. **60**:428, 1937.

The retarded coagulation observed in the blood of six rabbits and eleven dogs immediately after anaphylactic shock was regularly associated with increased amounts of antithrombin in the blood. The antithrombic activity was as much as one hundred times the normal. The fibrinogen content of the plasma was not significantly affected. For the reasons cited in the text, there is reason to believe that even the plasmas completely noncoagulable by calcium and tissue extract nevertheless contained sufficient prothrombin to effect coagulation; and although the platelet count was usually decreased after anaphylactic shock, the amount remaining would ordinarily have sufficed to cause coagulation within approximately normal limits of time. The increased antithrombic activity of the blood after anaphylactic shock is apparently the primary cause of the observed delay in coagulation.

FROM AUTHORS' SUMMARY.

ACTION OF IMMUNE SERUM ON HUMAN INFLUENZA VIRUS IN VITRO. T. P. MAGILL and T. FRANCIS JR., J. Exper. Med. **65**:861, 1937.

Studies have been conducted on the effect of immune serum on a strain of human influenza virus (PR8) grown in chick embryo tissue culture medium. The results have demonstrated (*a*) that when cells are exposed to the action of immune serum of high titer and subsequently washed freely they support the growth of virus as well as do cells treated with normal serum; (*b*) that, in agreement with the results of other workers, when virus is added to cell suspensions before the addition of immune serum of low titer it survives in the cells; (*c*) that when mixtures of immune serum of low titer and virus are added to cells, there is little evidence of survival or multiplication of the virus. Furthermore, when immune serum of high titer is used, the virus is inactivated regardless of whether the cells are first exposed to virus or to immune serum. Finally, virus mixed with a strong immune serum is inactivated in the absence of cells, as shown by the fact that centrifugation at high speeds of such serum-virus mixtures yields no active virus, whereas mixtures of normal serum and virus yield fully active virus.

FROM AUTHORS' SUMMARY.

CHEMICAL STUDIES ON BACTERIAL AGGLUTINATION. M. HEIDELBERGER and E. A. KABAT, J. Exper. Med. **65**:885, 1937.

By the application of an absolute, quantitative microchemical method for the estimation of agglutinins, precise data have been obtained on the course of the agglutination of type I pneumococci by homologous anticarbohydrate. Within the limitations imposed by the necessity for the agglutination reaction to take place at the bacterial surface, the reaction is shown to be analogous to the precipitin reaction and subject to the same laws. The entire process of a typical instance of specific bacterial agglutination has been quantitatively accounted for on a purely chemical basis and expressed in the form of equations derived from the law of mass action. Experimental verification of predictions based on the theory has shown a fundamental difference between this instance of specific bacterial agglutination and the commonly adduced analogies, and necessitated a revision of current conceptions regarding the role of electrolytes and of physical forces in the reaction.

FROM AUTHORS' SUMMARY.

LYMPH NODES AS A SOURCE OF THE NEUTRALIZING PRINCIPLE FOR VACCINIA. P. D. MCMASTER and J. G. KIDD, J. Exper. Med. **66**:73, 1937.

An antiviral principle is elaborated within the regional lymph nodes draining skin into which vaccinia is injected. The immunity conferred by clinical Jennerian vaccination may have its origin largely in the lymph nodes.

FROM AUTHORS' SUMMARY.

MECHANISM OF THE LYSIS OF PNEUMOCOCCI BY FREEZING AND THAWING, BILE AND OTHER AGENTS. R. J. DUBOS, J. Exper. Med. **66**:101, 1937.

Pneumococci, living or dead, are soluble in bile when (*a*) the autolytic enzymes are still present in a potentially active form, and (*b*) conditions are favorable for enzymatic action. Solubility of pneumococci in bile involves as a necessary step one or a few of the many stages of the autolytic complex. These observations hold true for the disruption of pneumococci by freezing and thawing, by previous desiccation with cold acetone and by dilute solutions of antiseptics. A possible mechanism is discussed to account for these forms of lysis.

FROM AUTHOR'S SUMMARY.

EFFECT OF THE BACTERIOLYTIC ENZYME OF PNEUMOCOCCUS ON THE ANTIGENICITY OF ENCAPSULATED PNEUMOCOCCI. R. J. DUBOS, J. Exper. Med. **66**:113, 1937.

Mice immunized with heat-killed virulent pneumococci (type I) that have been treated with active preparations of the bacteriolytic enzyme acquire a certain degree of type-specific resistance to subsequent infection. This active immunity, however, appears to be due to the small amount of free acetyl polysaccharide present in the suspension of digested bacteria and is always less pronounced than that obtained with intact heat-killed cells. When subjected to the action of active preparations of the bacteriolytic enzyme, virulent pneumococci that have been killed by heat or iodine lose the antigenic property of stimulating in rabbits the formation of precipitating antibodies for the type-specific polysaccharide. The enzyme prepared from S or R pneumococci, irrespective of the type from which these may be derived, is equally effective against the capsular polysaccharide antigen of any specific type of this bacterial species. The inactivation of the capsular polysaccharide antigen is observed when the cells are merely rendered gram-negative, without being caused to undergo actual disintegration or lysis. These observations emphasize the importance of minimizing the chances of alterations due to the action of cellular enzymes in the course of preparation of the cell suspensions to be used as immunizing agent.

FROM AUTHOR'S SUMMARY.

THE GONOCOCCUS COMPLEMENT FIXATION TEST. A. COHN, J. Lab. & Clin. Med. **22**:627, 1937.

The production of complement-fixing antibodies for the gonococcus is dependent on the duration and spread of the infection. In cases of acute complicated gonorrhea the complement fixation test is strongly positive, while in cases of gonorrhea of especially long standing the reaction is sometimes only weakly positive. Consequently + and + + reactions must be regarded with suspicion. The complement fixation test is an aid in the determination of cure if a previously positive reaction becomes negative, and the clinical and bacteriologic findings are also negative. The negative reaction by itself does not mean the absence of gonococci. A positive reaction persisting longer than one year after a clinical and bacteriologic cure is a strong indication that a latent focus of the gonococcus is present. Further examinations should be carried out in order to detect the focus.

FROM AUTHOR'S SUMMARY.

THE TYPE OF CLOSTRIDIUM WELCHII IN HUMAN FECES, WITH SPECIAL REFERENCE TO PERNICIOUS ANEMIA. G. R. BORTHWICK and J. D. A. GRAY, Brit. J. Exper. Path. **18**:119, 1937.

All of five strains of Clostridium Welchii isolated from the feces of patients suffering from pernicious anemia produced toxins of the A type, as shown by the short period of incubation required to produce the optimal concentration of toxin and the neutralization of the toxins by any of the four standard type antitoxins. Of five strains from the feces of healthy persons, two were either completely or practically nontoxic, and the other three produced toxins of the A type. Differ-

ences between the toxins of the strains from the two sources could therefore not be elicited other than in the degree of toxicity. Toxin, when produced in amounts suitable for testing, was consistently of type A. Evidence therefore is still lacking that man harbors Cl. Welchii of types other than type A.

FROM AUTHORS' SUMMARY.

THE AGGLUTINATION OF BACILLUS TYPHOSUS BY TRYPAFLAVINE AND ITS RELATION TO THE VI ANTIGEN. W. HIRSCH, J. Path. & Bact. **44**:349, 1937.

Suspensions of perfectly smooth cultures of Bacillus typhosus are agglutinated by trypaflavine. This reaction is closely linked up with the Vi antigen of this organism. Saline washings from sterile agar slopes, containing agar in colloidal form, are also flocculated by trypaflavine. The colloidal agar present in suspensions prepared by washing off agar cultures modifies the trypaflavine agglutination of smooth and rough culture of B. typhosus in different ways. The differentiation by the trypaflavine test of smooth and rough variants of B. typhosus is possible only by eliminating the disturbing effect of the Vi antigen, which most strains of typhoid bacilli possess.

FROM AUTHOR'S SUMMARY.

## Tumors

EFFECT OF INJECTING STARCH GRAINS INTO TRANSPLANTED TUMORS. R. CHAMBERS and C. G. GRAND, Am. J. Cancer **29**:111, 1937.

The injection into mouse tumors of starch grains, which are positively chemotactic to leukocytes, occasions a marked accumulation of polymorphonuclear leukocytes in and around the tumor. The accumulation definitely inhibits or slows down further growth of the tumor and in many cases results in complete regression of the tumor through a process of aseptic necrosis and subsequent resorption of the necrotic material.

FROM AUTHORS' SUMMARY.

CARCINOGENIC ACTIVITY OF THE CHOLANTHRENES AND OF OTHER DERIVATIVES OF 1,2-BENZANTHRACENE. L. F. FIESER and others, Am. J. Cancer **29**:260, 1937.

While the important problem of determining the manner in which certain hydrocarbons of the 1,2-benzanthracene series initiate malignant growth remains unsolved, the clear definition of the structural features which are essential for the development of high carcinogenic potency may clarify the problem and point the way to its solution. The recognition that cancer-producing power of a high order is associated with simple meso-alkyl 1,2-benzanthracenes opens various new lines of investigation, as does the discovery of carcinogenic properties in 3-hydroxy-1,2-benzanthracene and its methyl ether. The discovery of substances far simpler in structure than methylcholanthrene which approach this compound in carcinogenic potency has a possible bearing on the hypothesis that cancer-producing hydrocarbons may arise in the organism by abnormal metabolism of cholesterol or of bile acids. Hitherto this hypothesis has found some support not only in the demonstration that bile acids can be transformed by chemical means into methylcholanthrene but also in the apparently striking circumstance that this particular hydrocarbon, which carries as a mark of its possible origin the five-membered ring characteristic of the sterols and gonadotropic substances, is outstandingly potent in comparison with all previously known compounds except cholanthrene. Since the investigation of the possible biologic formation of methylcholanthrene by direct experimentation presents unusual difficulties, the circumstantial lines of evidence have been of considerable importance in molding current views. The present results indicate, however, that the high potency of methylcholanthrene is due chiefly to the fact that the hydrocarbon is a derivative of 1,2-benzanthracene having an alkyl substituent in the 10 position, and that similarly constituted hydrocarbons of far simpler structure share its remarkable carcinogenic activity. 10-methyl-1,2-benzanthracene and 5,10-dimethyl-1,2-benzanthracene lack both the cyclopentano

ring and the $C_{20}$-methyl group characteristic of the sterols and bile acids, and there is at present no good reason for supposing that these hydrocarbons can arise in the body. It is still entirely possible that methylcholanthrene is involved in the incidence of some forms of spontaneous cancer, but Fieser and his co-workers feel that their results weaken somewhat the purely circumstantial evidence pointing in this direction.

FROM AUTHORS' SUMMARY.

CARCINOMA OF THE LIVER IN MICE FOLLOWING INJECTION OF 2-AMINO-5-AZOTOLUENE. M. J. SHEAR, Am. J. Cancer **29**:269, 1937.

Subcutaneous administration of 2-amino-5-azotoluene ("o-amido-azo-toluol") in mice of a pure strain gave rise to multiple primary liver-cell carcinoma in every animal that survived for one year. Several of the hepatic tumors were successfully transplanted subcutaneously and retained their hepatic cell character after repeated transplantation. Similar transplantation of normal liver tissue gave negative results.

FROM AUTHOR'S SUMMARY.

TRANSPLANTATION OF TUMOR CELLS TO NORMAL AND PREIRRADIATED HETEROLOGOUS ORGANISMS. J. CLEMMESEN, Am. J. Cancer **29**:313, 1937.

Crocker mouse sarcoma 180 when inoculated into rats previously irradiated with from 500 to 600 roentgens produces transient growths, though of greatly varying size, in all animals still alive on the fourteenth day. The successful achievement of serial passages through a period of eighty-five days proves that irradiation with roentgen rays creates conditions for the growth of tumors in a heterologous organism superior to the conditions in normal rats. Clemmesen suggests that this effect of exposure to roentgen rays, which has its fully authenticated parallels in relation to homologous tumors and leukoses, is due to a postponement of the mobilization of the defense against foreign cells in general.

FROM AUTHOR'S SUMMARY.

LACK OF ESTRIN CONCENTRATION IN MAMMARY ADENOFIBROMA OF RATS. F. C. MOHS, Am. J. Cancer **29**:356, 1937.

Extraction and assay of spontaneous and transplantable adenofibroma of the rat mammary gland failed to demonstrate a high content of estrogenic substance. Flooding of the organism with estrogen for two to three weeks prior to putting the animal to death failed to increase the estrogen of these tumors or of the control striated muscle to a demonstrable level. Only by injection of extremely high doses of an estrogenic substance for two or three days prior to putting the animal to death was it possible to bring the estrogen of the tumors to demonstrable levels, and then the level was of the same order as that found in the control muscle tissue. Hence, adenofibroma does not owe its power of growth to an ability to concentrate estrogen.

FROM AUTHOR'S SUMMARY.

SOLITARY CUTANEOUS AND SUBCUTANEOUS LEIOMYOMA. A. P. STOUT, Am. J. Cancer **29**:435, 1937.

Fifteen cases of solitary leiomyoma are reported. Four were cutaneous. Eleven were subcutaneous. A review of the literature shows that at least 85 cases of solitary and 132 cases of multiple cutaneous and subcutaneous leiomyoma have been recorded. A complete bibliography of these is appended. The salient clinical features of the solitary tumors include their generally long duration and small size, their peculiar distribution, especially on the extensor surfaces of the upper and lower extremities, the scrotum, the labium majus, the nipple and areola, the cheeks and rarely elsewhere, and the characteristic pain, often paroxysmal, frequently caused by them. That this pain is probably associated with violent contractions of the neoplastic smooth muscle is indicated by the observations of a number of reporters. Morphologically the tumors are composed chiefly of smooth

muscle derived from one or another of the smooth muscle structures in the areas involved, and they develop in two chief forms, one without and the other with peculiar vascular structures, which are probably in the nature of veins. The failure to find any neurites in any of the tumors reported here is admitted, and the fact that one observer, Grybowski, succeeded in staining a large number in one cutaneous leiomyoma is regarded as suggestive that the attempts of the author and others have failed because of technical errors. The opinion is expressed that these tumors, together with the tumors of the neuromyo-arterial glomus, probably form the bulk of the painful subcutaneous tubercles (tubercula dolorosa) of the older writers of the eighteenth and nineteenth centuries, and suggestions for differential diagnosis are made. The rarity of malignant cutaneous and subcutaneous leiomyomas is pointed out, and the effectiveness of surgical excision as the treatment of choice for the leiomyomas is stressed. Finally, it is suggested that these solitary tumors, contrary to the general impression, are as common as, if not more common than, the multiple cutaneous leiomyomas and that a wider knowledge among clinicians of their existence and a more general use of differential fiber staining in pathologic laboratories will lead to their more frequent recognition.

FROM AUTHOR'S SUMMARY.

SERCLOGIC OBSERVATIONS ON REGRESSION OF IMPLANTS OF JENSEN'S RAT SARCOMA. T. LUMSDEN and H. J. PHELPS, Am. J. Cancer **29**:517, 1937.

The serum of seventy rats into which fragments of Jensen rat sarcoma had been implanted has been examined frequently. Certain characteristic cytotoxins frequently appeared in the serums of these rats. If the serum of any particular rat showed a titer of these cytotoxins in excess of 2 per cent, the tumor in that rat invariably regressed. The serum of every rat in which a tumor was regressing contained at some time or other a titer of cytotoxins varying from 2 per cent to 80 per cent (average, 17.7 per cent). If the tumor was progressing the serum of the rat never contained a titer of over 2 per cent (average, 0.4 per cent). The observations suggest that the production of these cytotoxins is part, at least, of the mechanism by which spontaneous regression of tumors takes place.

FROM AUTHORS' SUMMARY.

GENETICS OF CANCER OF THE BREAST. R. P. MARTYNOVA, Am. J. Cancer **29**:530, 1937.

Hereditary factors play a definite role in predisposition to cancer of the breast. Predisposition to cancer of the breast is in some way connected with predisposition to cancer in general. A hypothesis of monohybrid recessive inheritance of cancer of the breast is rejected. The questions of the number of involved genes, their mode of inheritance and the relative influence of the environment conditioning their expression are still open questions.

FROM AUTHOR'S SUMMARY.

OSTEOGENIC SARCOMA OF THE FEMUR IN A GUINEA PIG. S. A. LEADER, Am. J. Cancer **29**:546, 1937.

To the twenty recorded instances of a spontaneous tumor in the guinea pig one is added, that of an osteogenic sarcoma of the femur with pulmonary metastases and a pathologic fracture of the corresponding tibia and fibula. The primary site of the only other recorded osteogenic sarcoma was unfortunately not discovered.

FROM AUTHOR'S SUMMARY.

RELATIVE INCIDENCE OF OOPHORECTOMY IN WOMEN WITH AND WITHOUT CARCINOMA OF THE BREAST. W. E. HERRELL, Am. J. Cancer **29**:659, 1937.

Something is known of the role of the ovary in the development of spontaneous mammary adenocarcinoma in mice. A review of 1,906 records of women treated

for carcinoma of the breast and 1,011 records of women in a similar age group without mammary carcinoma discloses that in the cancer-bearing group the incidence of complete oophorectomy or castration before carcinoma was diagnosed was 1.5 per cent. The incidence of castration in the noncancerous group was 15.4 per cent, or ten times as great. These findings warrant further study in this field.

FROM AUTHOR'S SUMMARY.

PINOCYTOSIS BY MALIGNANT CELLS. W. H. LEWIS, Am. J. Cancer **29**:666, 1937.

Malignant cells from rat and mouse sarcoma often show in tissue cultures active, wavy, ruffle pseudopodia, which engulf complex fluid mediums containing proteins and other substances that cannot diffuse into them. The fluid enters the cells as globules which move centrally. The contents are digested and the fluid then diffuses out of the cell. Thus many sarcoma cells may at times exhibit considerable intracellular digestion. Pinocytosis by malignant cells is similar to pinocytosis by normal macrophages.

FROM AUTHOR'S SUMMARY.

DEVELOPMENT OF TUMORS IN FEMALE MICE TREATED WITH 1,2,5,6-DIBENZANTHRACENE AND THEELIN. I. H. PERRY and L. L. GINZTON, Am. J. Cancer **29**:680, 1937.

A report is made of the production of neoplasms in a colony of female mice, half of which were treated with 1,2,5,6-dibenzanthracene and the other half with 1,2,5,6-dibenzanthracene and theelin. Numerous benign epithelial proliferations of the skin, breast, uterus, alimentary tract and lungs occurred before the development of carcinoma and appear to be causally related to the subsequent malignant growths. The incidence of carcinoma of the skin is chronologically related to the development of carcinoma of the breast. Carcinoma of the breast is causally and chronologically related to carcinoma of the uterus.

FROM AUTHORS' SUMMARY.

THE ELEMENTARY BODIES OF INFECTIOUS MYXOMATOSIS OF RABBITS. T. M. RIVERS and S. M. WARD, J. Exper. Med. **66**:1, 1937.

From the results of the experiments described in this paper it is obvious that large amounts of elementary bodies of myxoma can be obtained in a relatively pure state by the methods used. Furthermore, it is evident that infectious myxomatosis is a viral disease in which elementary bodies of the same order of magnitude as vaccinal elementary bodies play a conspicuous role in that they either represent the etiologic agent or are intimately associated with it. The bodies are specifically agglutinated by antimyxoma serum and are agglutinated to a less extent by serum from rabbits convalescing from fibroma, a disease closely related to myxoma. In virus-free filtrates of emulsions prepared from infected skin there is a soluble precipitinogen or precipitinogens specific for the malady. Moreover, a specific precipitinogen or precipitinogens are demonstrable in virus-free serum of animals acutely ill as a result of extensive infection with myxoma virus. It is believed that this is the second viral disease, yellow fever being the first, in which a specific soluble antigen free from virus has been found in the serum of ill animals.

FROM AUTHORS' SUMMARY.

PROPERTIES OF THE CAUSATIVE AGENT OF A CHICKEN TUMOR. A. CLAUDE, J. Exper. Med. **66**:59, 1937.

The agent causing chicken tumor I can be separated from the other constituents of the tumor filtrate by means of high speed centrifugation. The separation was practically complete when a filtrate of average viscosity (0.018 poise) was submitted to a centrifugal field of 14,000 times gravity for two hours. Relative purification of the agent was obtained by means of differential centrifugation and

washing in Tyrode's solution or in distilled water. The washed sediment gave opalescent solutions composed of minute particles of approximately but not exactly the same size. The dry weight of the active material separated by high speed centrifugation was 0.0008 mg. per cubic centimeter of filtrate or about 1 part per 2,800 parts of the total filtrate. The tumor-producing activity of the washed sediment was significantly greater than that of the entire original filtrate. It is suggested that the gain in tumor-producing power was effected by removal of an inhibiting factor known to occur normally in extracts of chicken tumors.

FROM AUTHOR'S SUMMARY.

CARCINOGENIC ACTION OF DIBENZANTHRACENE ON LUNGS OF MICE. H. B. ANDERVONT, Pub. Health Rep. **52**:212, 1937.

Mice of strain A were given subcutaneous injections of a lard solution of dibenzanthracene. In these animals more tumors of the lung developed than subcutaneous tumors, and the tumors in the lungs arose earlier than did the subcutaneous growths. Hence it appears that the lungs of these mice were more delicate test objects than the subcutaneous tissues for the carcinogenic activity of 1,2,5,6-dibenzanthracene.

The reason for the appearance of tumors in the lungs of mice painted with tar or given injections of carcinogenic compound is unknown. The observations recorded in this paper indicate that in mice of strain A the response in the lungs occurred in a relatively short period of time. While it does not appear likely that 0.8 mg. of 1,2,5,6-dibenzanthracene in 0.2 cc. of lard was capable of altering the state of a mouse to such an extent that tumors of the lungs arose much earlier than under normal conditions, it is not impossible that very small amounts of a carcinogenic agent left the site of injection and came into contact with the tissues of an organ which is known to be extremely susceptible to tumor growth.

INFLUENCE OF HEREDITY ON LUNG TUMORS INDUCED BY SUBCUTANEOUS INJECTION OF A LARD-DIBENZANTHRACENE SOLUTION. H. B. ANDERVONT, Pub. Health Rep. **52**:304, 1937.

Female mice of strain A, susceptible to both spontaneous and induced tumors of the lungs, were bred to males of strain C 57 black, known to be resistant to spontaneous growths. Conversely, females of strain C 57 black were mated to males of strain A. The females of the first hybrid generation were bred to their brothers to procure the second hybrid generation.

Most of the first hybrid generation of each outcross and approximately half of the second were given subcutaneous injections of a lard solution of dibenzanthracene. Absence of growths in the lungs in a high proportion of the controls (not given injections) of both the first and second hybrid generations shows that the pulmonary nodules in the mice given injections did not arise spontaneously.

The progenies of the first hybrid generations from the two outcrosses were equally susceptible to the carcinogenic action of dibenzanthracene. An analysis of the results in the second hybrid generation failed to reveal any influence exercised by sex, pedigree or color on the occurrence of the induced growths. The presence of tumors of the lungs in a high percentage of the first and second hybrid generations indicates that the susceptibility of the lungs to tumors induced by subcutaneous injections of a lard solution of dibenzanthracene is inherited in a dominant manner.

R. J. LEBOWICH.

TRANSPLANTABLE CARCINOMA OF FOWL WITH DISCUSSION OF THE HISTOGENESIS OF MIXED TUMORS. L. FOULDS, J. Path. & Bact. **44**:1, 1937.

A fowl carcinoma, believed to have originated in the oviduct, has been transmitted through twelve generations by cellular grafts. There was an initial period of difficult transmission terminated at the sixth passage by an abrupt improvement which was maintained until recently, when transmission again became difficult.

The histologic structure of the original growth was reproduced in tumors of the first generation but subsequently was greatly modified. Bone and cartilage were frequently present, and the tumors then had a "mixed" structure. Stages in the formation of bone were traced. It was concluded that the bone was formed by the hosts' connective tissue cells, which were constrained to differentiate in this way by conditions brought about by the epithelial parenchyma. The close resemblance between the fowl carcinoma and mixed tumors of the human salivary glands is described and discussed. It is concluded that in this type of mixed tumor only the epithelial component is neoplastic.

FROM AUTHOR'S SUMMARY.

VITAL STAINING IN EXPERIMENTAL CARCINOGENESIS IN MICE. J. W. ORR, J. Path. & Bact. **44**:19, 1937.

White mice have been stained intra vitam with an indicator dye, phenol red, during the progress of experimental carcinogenesis, the cancer-producing agents being tar, dibenzanthracene and benzpyrene. The earliest change was an increase in the intensity of staining throughout the area treated with the carcinogen, the color remaining red but of a considerably deeper shade than that of the normal skin. At a later stage foci in the treated skin stained yellow instead of red, and at the same time tumors began to appear. In some cases the whole treated area became orange or mottled with yellow. The progress of the staining phenomena in the case of each of the carcinogens was closely associated with the rate of induction of tumor. A high proportion of the tumors first appeared in relation to yellow areas. It is suggested that the yellow coloration is an indication of local functional ischemia. Various other interpretations have been considered. Rapid growth of tumors was in general associated with disappearance of the yellow color, while the persistence of this color was frequently accompanied by retardation or actual regression of warts. In control observations with noncarcinogenic irritants and spontaneous lesions it was found that yellow staining might occur around actively healing ulcers.

FROM AUTHOR'S SUMMARY.

THE ETIOLOGY OF BENIGN ENLARGEMENT OF THE PROSTATE IN THE DOG. S. ZUCKERMAN and J. R. GROOME, J. Path. & Bact. **44**:113, 1937.

Of nine prostates of mature dogs studied, only one was normal in size and histologic structure. Seven of the remaining eight were enlarged, the enlargement being due to simple glandular hyperplasia, possibly determined by an excess of androgen. The ninth specimen was greatly and irregularly enlarged. In histologic appearance it was different from the other specimens but was identical with the prostates of dogs which had been treated with an estrogenic substance (theelin). This correspondence is taken as direct evidence that enlargement of the prostate may result spontaneously from excessive estrogenic stimulation.

FROM AUTHORS' SUMMARY.

DIAGNOSIS OF CANCER. E. SALAMON, Ann. Inst. Pasteur **57**:299, 1936.

A diagnostic method based on modification of the coagulating power of leukocytic and erythrocytic extracts was proposed by Mendeléeff in 1934. Tests on patients who had been proved to have cancer and on controls indicated that no false results occur when the patients tested are noncancerous but that a number of failures may occur when the patients tested have cancer. The test is undergoing modification but is considered to be of value.

M. S. MARSHALL.

INTRACUTANEOUS EPITHELIOMA OF RABBITS. A. BESREDKA and L. GROSS, Ann. Inst. Pasteur **57**:343, 1936.

Injection of normal brain into the skin of a rabbit protected it against an inoculation of epithelioma in the skin but not against one elsewhere, whereas inoculation of epithelioma in the skin immunized specifically against inoculation

of tumor in any part of the body. Spontaneous resorption of the tumors was irregular except when the neoplastic material was injected intracutaneously, so that this method provided immunity with maximal security. No antibodies were demonstrable, and the immunity was regarded as local.

ACTION OF PHYSICAL AGENTS ON EHRLICH'S SARCOMA. A. BESSEMANS and L. ASAERT, Ann. Inst. Pasteur **57**:516, 1936.

A study of the Ehrlich sarcoma of white mice revealed the following: Virulence disappeared after two days at 17 C. or four days at from 6 to —10 C., but survived the latter temperature for three days; virulence survived from five to thirty minutes at from 50 to 60 C., but was reduced; tumors progressed in animals exposed to 42 C. for fifty-eight hours or irradiated; the virulence of sarcomatous material also survived irradiation, except when this was coupled with excessive heat.

M. S. MARSHALL.

COPPER METABOLISM AND EXPERIMENTAL CANCER IN RATS. S. SÜMEGI, Frankfurt. Ztschr. f. Path. **48**:35, 1935.

This investigation was conducted to determine whether or not during the growth of a tumor the metabolism of copper is affected, and if so, whether such damage can be corrected by parenteral administration of copper. Rats in which the Ehrlich-Putnoky mouse carcinoma was successfully transplanted were used for the experiments. It was found that during the growth of the tumor the metabolism of copper is distinctly damaged. The copper is stored in the tumor itself and in the liver, and is thus unavailable for metabolism by the body. Under these conditions the stomach contains 40 per cent less copper than normally, and therefore the formation of the antianemic factor in the stomach is inhibited. The storage of copper in the tumor is explained on the supposition that copper is a catalyst in "anoxybiotic breathing." The storage of copper in the liver is the result of damage to the liver parenchyma. Parenteral medication of copper improves the metabolism of copper and the anemia.

OTTO SAPHIR.

NEUROFIBROMA OF THE STOMACH. O. SPÜHLER, Frankfurt. Ztschr. f. Path. **48**:149, 1935.

Spühler reports ten cases of neurofibroma of the stomach and reviews twenty-three cases from the literature. In none of the cases did the patient show evidence of generalized neurofibromatosis. The age of the youngest patient was 59 years. In eight instances the tumor was situated in the musculature of the stomach and encroached on the muscle bundles to such a degree that portions of the tumor which projected into the lumen were not covered by muscle tissue. In one instance the tumor was situated in the submucosa. There was an increase of ganglion cells in the plexus in the immediate vicinity of the tumor. The author assumes that this type of tumor originates from the plexus because it is intramuscular and because portions of Auerbach's plexus and also definite ganglion cells are found in it. It is suggested that the stem cell is a structure related to the sympathetic nervous system. The author also stresses the frequent association of the neurofibroma with other changes in the stomach. Of nine stomachs, four showed ulcers and one multiple erosions.

OTTO SAPHIR.

CARCINOIDS OF THE SMALL INTESTINES. J. GIERLICH, Frankfurt. Ztschr. f. Path. **48**:202, 1935.

The carcinoids of the small intestine are small tumors, usually occurring in advanced age. The architecture is characteristic and can be compared to that of the organs of internal secretion. A relationship to the endocrine system is therefore suggested. The cytoplasm of the tumor cells contains doubly refractible lipoids and argentaffin and chromaffin granules. Malignant carcinoids are generally purely

argentaffin. It is assumed that carcinoids originate from displaced embryonal structures. The mother cells probably are the enterochrome cells, which show chromaffinity and argentaffinity as do the carcinoid cells. It is assumed that chronic inflammatory stimuli cause continual regeneration of these cells and thus prepare the ground for the origin of the tumors. The fact that a high percentage of patients with carcnoid tumors also have other types of tumors, benign or malignant, as well as various embryonal malformations, deserves special attention.

OTTO SAPHIR.

CARCINOMA ARISING IN REMARKABLY SHORT TIME. A. KORÉNYI, Frankfurt. Ztschr. f. Path. **48**:314, 1935.

Two cases of rapid inception of carcinoma following trauma are reported. A chimney sweep aged 31 sustained a superficial injury involving abrasion of the skin in a small area. Ten days later an enlargement of the area was noted, and six weeks later a basal cell carcinoma was diagnosed. Microscopically, the skin at the site of the carcinoma was intact, and no evidence of injury was discernible. The time of appearance of the carcinoma was considered to be from eight to ten days after the injury. A 54 year old man was struck with a pipe at the site of a pedunculated tumor of the skin, which was reputedly from 4 to 5 years old. The trauma caused no loss of substance. An immediate increase in the size of the tumor ensued, and four weeks later a superficial erosion was noted and diagnosed as basal cell carcinoma.

OTTO SAPHIR.

PATHOGENESIS OF PULMONARY CARCINOMA. K. LINDBERG, Arb. a. d. path. Inst. d. Univ. Helsingfors **9**:1, 1935.

Lindberg devotes considerable space to the subject of epithelial metaplasia and its relation to precancerous and cancerous conditions. His observations are based on his study of forty-two primary carcinomas of the lung. All the lungs were cut once from front to back through the hilus and were then cut from top to bottom at 1 cm. intervals. Appropriate labeling made it possible to locate a lesion anywhere. In thirty-nine of the lungs, the bronchi of the lung containing the cancer were systematically examined. In twelve bodies, the entire opposite lung or its hilar portions were examined. Altered bronchial epithelium was found in one or more places in fifteen lungs (38 per cent). In the twelve bodies in which both lungs were examined, altered bronchial epithelium was encountered seven times and only twice in the lung which was not the site of the tumor. Metaplastic epithelium was found especially where bronchi branched. It was most common in lungs in which the cancer consisted of well differentiated squamous epithelium. It was not noticed in the lungs in which the cancer consisted of cylindric cells or was adenomatous.

The departure from normal was represented by three types of epithelium. The first showed a gradual transition from normal bronchial epithelium to stratified cylindric epithelium. The second was a transitional type in which the cells in the uppermost epithelial layers were rounded but not stratified. The third was a stratified epithelium with flat cells of varying grades of maturity in its outer layer. The degree of undifferentiation in the cells and their tendency to invade various layers of the bronchial lining were regarded as indexes of malignancy.

There are 557 photomicrographs to illustrate the various phases of malignant change in the lungs examined.

GEORGE J. RUKSTINAT.

A PECULIAR CASE OF HEREDITARY CANCER LIMITED TO ONE SEX. H. FEDERLEY, Finska läk.-sällsk. handl. **78**:241, 1935.

In the course of cultivation of the butterfly Pygaera pigra 39 cocoons of the I variety remained alive, while all those of the Ue variety perished. In 128 caterpillars the cause of death was found to be a tumor. No tumor was found in individuals of the I variety. The tumor was encountered only in males but was

transmitted by the females. Federley explains the behavior of this tumor by assuming that a mutation in the chromosomal pattern of the polar body makes it persist in the female, while in the male the different chromosomal formula permits not only the persistence but even the multiplication of the polar body. Its rapid multiplication leads to the formation of tumors. These were observed in the intestinal tract, testicle, ganglions, skin and trachea.

I. DAVIDSOHN.

## Medicolegal Pathology

DETECTION OF ISO-AGGLUTININS IN BLOOD STAINS. E. BALGAIRES and L. CHRISTIAENS, Ann. de méd. lég. **17**:215, 1937.

The authors studied the length of time that iso-agglutinins persist in blood stains. Higuchi found that they disappeared after six months; Lattes found them still present after eighteen months.

Forty-six blood stains were examined from time to time for periods up to ninety days to determine the effect of aging alone on the iso-agglutinins. At the end of the three month period, in only a small minority of the stains were the agglutinins no longer demonstrable. In fifteen of twenty group O blood stains both agglutinins were still demonstrable after ninety days; the same was true in fifteen of nineteen group A blood stains and in all of seven group B stains.

Thirty-seven other blood stains were studied in order to determine the combined effect of sunlight and aging on the iso-agglutinins. Even under these circumstances the iso-agglutinins were still demonstrable in more than one half of the stains at the end of three months. These experiments confirm Hirszfeld's opinion that the failure to demonstrate the presence of iso-agglutinins in a blood stain has no conclusive value. However, they should be looked for in every case, since group O is the most common group, and if both anti-A and anti-B were found, it would be possible to draw definite conclusions as to the group of the stain.

A. S. WIENER.

SECTIONING THE BRAIN FROM A DISINTERRED BODY. G. PANNING, Deutsche Ztschr. f. d. ges. gerichtl. Med. **28**:178, 1937.

The body of a man who had suffered from epileptic seizures following an injury of the head was found on a farm, face down in the mud, three weeks after death. Because of marked decomposition the usual methods of examination were not employed. The head was frozen in a mixture of four parts of ice and one of salt, for twenty-four hours. By sawing through the frozen skull and brain, a scar was encountered in the right temporal lobe, and in the leptomeninges and dura in this area hemosiderin was found. Calcification of the ganglion cells of the underlying brain was demonstrated.

GEORGE J. RUKSTINAT.

THE POWDER STAIN ABOUT BULLET WOUNDS. H. ELBEL, Deutsche Ztschr. f. d. ges. gerichtl. Med. **28**:359, 1937.

Five shots fired from the same gun into white cloth and into fresh corpses were used to produce the powder marks studied. The intensity of powder marks is in general determined by the condition of the weapon. When several shots are fired by one weapon, the marks change but not sufficiently so that one can determine the order in which the shots were fired. The stain is dependent on the amount of material in the barrel of the gun; there is a likelihood that more of this material will be present when several shots are fired and when the gun is oiled.

Infra-red photography was serviceable in bringing to light powder stains on dark and bloody surfaces and on dried skin. These marks are in a zone from 1 to 2 mm. wide in skin or clothing and occur when shots are fired either near at hand or from a distance. Such stains may even be revealed on dark wool by infra-red plates.

GEORGE J. RUKSTINAT.

INFRA-RED PHOTOGRAPHY OF WOUNDS OF ENTRANCE AND EXIT. S. MANCZARSKI, Deutsche Ztschr. f. d. ges. gerichtl. Med. **28**:366, 1937.

Infra-red photography yields good results in establishing the presence of powder stains about entrance wounds of bullets fired from a distance when portions of clothing are carried into the wound. In wounds of the naked skin, the powder stain is found in the region of the dried epidermis. Its presence can be demonstrated by microscopic, chemical, spectroscopic and roentgenologic tests. The powder smudge itself cannot be demonstrated by infra-red photography. The method, however, is valuable in demonstrating bloody extravasations in the region of the wounds. The infra-red rays which pass through the cornified layer of the skin are absorbed by the hemorrhage and thus a dark spot with smeared contours appears about the entrance wound. The circumference of this spot indicates the extent of the bleeding, and neither the contour of the powder stain nor the wound itself is visible. In the vicinity of exit wounds dark spots are also encountered but are less widespread.

GEORGE J. RUKSTINAT.

# Society Transactions

## NEW YORK PATHOLOGICAL SOCIETY

N. Chandler Foot, *President*

*Regular Meeting, Oct. 28, 1937*

Milton Helpern, *Secretary*

Colostrum Cell Carcinoma. Andrea Saccone and Abraham Rosenthal.

This paper will be published in the *American Journal of Cancer.*

DISCUSSION

Francis Carter Wood: I have been much interested in this tumor because the paper was sent to the *American Journal of Cancer* for publication. As I have the habit of making careful studies of the material which is sent to me, usually getting slides of the specimen, I asked the authors of the paper to let me have fresh material. First, I was struck by the fact that the x-ray films of the skull and of the rest of the body are suggestive of myeloma, and I showed the films to two or three roentgenologists, who said "myeloma" without any hesitation. Second, I observed that the morphologic character of the tumor is curious. The growth does not correspond with any tumor of the breast that I have ever seen, or with any of the mammary tumors which are figured in the textbooks. So, when I obtained the fresh material, I had it stained, and there was no demonstrable fat in the cells, and no glycogen. The glycogen may have been dissolved in the solution of formaldehyde used as fixative, but there is no logical basis for the assumption that glycogen was present in the tumor cells in large quantity.

The fact that these cells are widely distributed in all the organs and bones and that they retain their type throughout while the morphologic changes of the bone marrow, especially, are so characteristic indicates that these cells are plasma cells. The colostrum cell is not a clear cell; it has a spongy structure and, true, a small nucleus; the colostrum cells still remaining in the ducts of this breast show the spongy structure and stainable fat. The widely distributed cells are perfectly clear. When stained with methyl green and pyronine they have green nuclei and red cytoplasm.

I think the case is also of interest because the history is peculiar for carcinoma. Bilateral carcinoma of the breast growing synchronously and of exactly the same morphologic character is unknown. The large cells in the ducts of the breast are not the same as the infiltrating ones. Metastases in the breast are unusual but are possible with myeloma. Certainly the title should not remain "Colostrum Cell Carcinoma," for the tumor cells are not colostrum cells. The growths may be clear cell carcinoma but in my opinion are not.

Paul Klemperer: Dr. Saccone and Dr. Rosenthal showed me sections before this meeting. I cannot help it—I was convinced and I still am convinced that this is carcinoma. I was not impressed by a resemblance of the tumor cells to plasma cells, but that is a point that could be discussed at length, and I am perfectly willing to submit my opinion to the judgment of the Committee on Microscopy. If the tumor were plasma cell myeloma, the case would be a most unusual one. Classic myeloma should in general be limited to the bones but is not always; yet I have never seen or read of a case in which there was such widespread extension into the breasts and lymph nodes.

In regard to the cells, I should object to calling them colostrum cells. I do not see why one should call them colostrum cells. What one finds in the mam-

mary tumors is carcinoma with a large amount of fat and glycogen. The finding is not so uncommon. I remember a specimen shown to me as one of malignant Grawitz tumor of the breast which was evidently carcinoma with a large amount of fat.

In regard to the x-ray picture, I remember having encountered two cases in which there was just as wide involvement in which the diagnosis was unquestionably carcinoma, based on a specimen about which the department of roentgenology was in great doubt. Because I am not a roentgenologist, I inquired from a roentgenologist whether it was possible to make a diagnosis between carcinomatosis of the bones and myeloma by roentgen examination and received an emphatic negative answer. This is certainly a most complicated case, and I think the Committee on Microscopy will have a pleasant time.

ARTHUR PURDY STOUT: I have nothing to add. With such conflicting opinions about the cells I do not think that I have any right, without studying them, to say anything except that as seen on the screen they do not remind me of plasma cells. However, they are so undifferentiated that I do not think I could tell.

ANDREA SACCONE: From the roentgenologic point of view I think that some approximate differentiation can be made from the location of the tumor in the bone, for usually myeloma is in the distal end of a long bone, and in the other case it is mostly in the proximal end of the long bone, but my knowledge of roentgenologic diagnosis does not permit me to go further.

We considered the case as one of carcinoma for three reasons: first, there was the histogenesis. I think there are some places in the original tumor of the breast in which one may find the origin in the ducts of the breast. The morphologic character of the cells has been carefully studied, and I think there is no question that one cannot regard these cells as myelomatous cells. Second, there is the question of metastases. As Dr. Klemperer has said, there are only two or three cases that I know of, recorded in the literature, in which a myeloma has metastasized, but even in those cases there was never such widespread metastasis. Third, there is the question of calling the tumor colostrum cell carcinoma. One can call it functioning cell carcinoma, because if one admits that it is carcinoma of the breast in that stage of hyperfunction of the breast which occurs after delivery, the cells that are spreading in the metastases are such typical cells that one can consider them only as cells from a functioning breast, and for that reason we used the name "colostrum cell carcinoma."

As to the question of staining fat, that is very important, because in many of the tumors there were some places in which the fat appeared to be present, and I have some slides from this case in which the fat is conglomerated in some areas of the section, but as Dr. Wood says, the cytoplasm does not really show the presence of fat. One must remember, however, that one is dealing with a tumor, and in a tumor it is not always possible to stain fat, because one is dealing with some abnormal product, with some type of abnormal fat that cannot respond to the usual stain.

[The sections of this tumor were submitted to the Committee on Microscopy, the members of which were Dr. Francis C. Wood, Dr. James Ewing and Dr. Alvin M. Pappenheimer. Dr. Wood's opinion is stated in the foregoing discussion; Dr. Ewing's was that the tumor was carcinoma, and Dr. Pappenheimer's follows: "I think it is carcinoma of the breast with unusually extensive metastasis and I cannot agree with Dr. Wood's suggestion that it is myeloma. The unusual feature of the tumor seems to be the numerous clear cells, apparently filled with glycogen of a type which I cannot recall having seen in tumor of the breast, but I do not think that this invalidates the diagnosis."]

EFFECT OF SUBCUTANEOUS INJECTIONS OF CONCENTRATED EXTRACT OF SPLEEN ON MOUSE SARCOMA 180. RICHARD LEWISOHN.

Two hundred and eighty-one animals with sarcoma 180 were treated by subcutaneous injection of concentrated extract of spleen in amounts of 0.5 cc. One cubic centimeter of the extract represents 100 Gm. of beef spleen. The extract

is an aqueous product prepared by extraction with water, adsorption with activated carbon, triple extraction with alcohol, and elution. Of these 281 animals, 60 per cent showed complete disappearance of the tumors. Of the corresponding 290 controls, only 8 per cent showed spontaneous regressions. Eighty animals were treated with other organ extracts. Twenty were treated with heart, liver, pancreas and testis, respectively, without effect on the normal growth of the tumors. Fifty-nine animals were given 0.1 cc. doses of the extract. These small doses stimulated the growth of the tumors even beyond that in the controls.

Twenty animals were given injections of merthiolate and twenty were treated with chlorobutanol, without effect on the tumors.

The injections were given near the left hip joint from seven to ten days after the transplantation of the tumor, depending on the size of the tumor. The tumor was near the right axilla. Marked hemorrhage was sometimes noted in the tumor after four hours, twenty-four hours or several days. Injections were given daily or on alternate days, as required. The tumor stopped growing, the hemorrhage increased and a scab developed which was ultimately expelled, leaving in its place a cavity with only a small border of tumor. Gradually the cavity grew smaller; the tumor tissue was absorbed and gradually disappeared, and the area where it had been presented an absolutely normal appearance. The tumors have not recurred during an observation period of over five months. Control mice showed marked loss of weight in spite of the rapid growth of the tumors. Mice treated with the extract began to gain weight before the tumors had disappeared and continued to gain after the disappearance of the tumors. The gain in weight was often an early indication that the tumors would disappear.

The fact that 0.1 cc. doses stimulate the growth of the tumors probably explains the failure of other authors to cause disappearance of malignant tumors with extracts of spleen, as all preparations of that organ on the market are less concentrated. The extract of spleen was tested with the help of Dr. Shwartzman and did not produce the Shwartzman phenomenon.

The spleens in tumor-bearing mice treated with the extract of spleen were often from four to five times as large as the spleens of normal mice or of those treated with other organ extracts.

Experiments now being performed in which the extract is being injected intravenously seem to indicate that it acts promptly on the tumor, without the hemorrhagic stage.

DISCUSSION

WILLIAM H. WOGLOM: Almost every day it is said that the use of transplantable tumors in therapeutic experiments is unjustifiable because so many agents will cause their disappearance, yet after twenty-five years of experience I am unable to name one single thing that can be depended on to make them recede in 50 per cent of the animals treated without detriment to the animals, or in 25 per cent. I therefore believe that the use of these growths is justifiable for what the mathematician would call a first approximation. If the experiment seems hopeful, it must be extended to other transplantable tumors and finally to spontaneous neoplasms and those caused by dibenzanthracene.

I have watched Dr. Lewisohn's experiment with sympathetic interest and can testify that all is as he has represented it to be. I cannot see that there is anything further to be said except, "Well done, thou good and faithful savant."

BENJAMIN N. BERG: How often did Dr. Lewisohn have to discard a control series of animals which showed spontaneous regression?

JACOB FURTH: I do not question the correctness of Dr. Lewisohn's findings, and all the members are interested to hear what he will do with spontaneous tumors, but the interpretation is of unusual interest. If I understood Dr. Lewisohn correctly, the enlargement of the spleen was due to the injections of the extract. Enlargement of the spleen in my experience is not significant in mice bearing spontaneous tumors, because histologic examination shows that it is due

to myeloid metaplasia and not to an increase in the specific splenic elements, and so may I ask what the histologic appearance of these large spleens was? Secondly, myeloid metaplasia of this type is more likely to occur in animals in which tumors have occurred and have undergone necrosis, and the common interpretation is that the enlargement is secondary to the necrosis or to the infection which is associated with necrosis, and I should like to ask, Does the injection of the extract induce enlargement of normal spleens in nontumor-bearing animals?

PAUL KLEMPERER: The histologic character of these spleens has been studied, but the study could not be finished in time to be presented here. I advised Dr. Lewisohn not to include the histologic description of these spleens in his report.

RICHARD LEWISOHN: I have stated in my paper that we discarded a few sets when the controls showed a high percentage of regressions. These sets were not included in the statistics.

Furthermore, I stated that control animals never showed enlargement of the spleen. An attempt is being made to study the size of the spleen in normal mice after these have received injections of spleen extract. These experiments are still unfinished.

CHRONIC INTRAHEPATIC OBLITERATING CHOLANGITIS. PAUL KLEMPERER.

This paper appeared in the *Journal of the Mount Sinai Hospital* (**4**:279, 1937).

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PAUL KLEMPERER, *Presiding*

*Regular Monthly Meeting, Nov. 18, 1937*

MILTON HELPERN, *Secretary*

ACTINOMYCOSIS OF THE OVARY. LIONEL S. AUSTER.

Actinomycosis in man occurs chiefly in the head, neck (especially in the jaws), lungs, skin and abdomen. The abdominal form most frequently is ileocecal. It is always of long course. It presumably gains its entrance through the intestinal tract or is inhaled, and is disseminated by the hematogenous route or by extension. Lymphatic involvement is limited to the secondary bacterial invaders of the intestine or associated organs. A primary ovarian involvement is unusual and gives a confusing clinical picture.

This 27 year old unmarried white woman had been ill for approximately two years, in the course of which she had been admitted three times to two hospitals. In August 1935, a "cold abscess" in the right half of the chest was drained by resection of a rib. There was prolonged discharge, necessitating a second operation three months later. In January 1936 she began to have diarrhea and fever, which continued for three weeks, with pain in the back, frequency of urination, chills and prolonged fever. With these symptoms she entered the Bronx Hospital on January 27. She also had complained of amenorrhea for two months, a loss of 25 pounds (11 Kg.) in weight in one year and premenstrual sensations without menstruation. Studies at that time showed a mass in the pelvis, including the uterus and parametric tissues, which under observation enlarged until it was within 2 fingerbreadths of the umbilicus. Sigmoidoscopy showed a picture suggesting receding bacillary dysentery, an observation which was seemingly confirmed by a blood titer of 1:320 against the Park-Hiss organism. Chemical examination of the blood and the Wassermann and Kahn tests gave negative results. The sputum was negative for tubercle bacilli; the Mantoux and Friedman reactions were negative. Pus from the healing wound disclosed no tubercle bacilli but disclosed Staphylococcus aureus. Roentgen examination showed the lungs to be clear. The

urine contained some pus and traces of albumin. It was thought that the patient had a hematometra. Dilatation and curettage, however, revealed none. It was then felt that the pelvic mass represented either tuberculosis or a neoplastic condition of the ovary, with uterine fibroid. An exploratory operation was advised. This was refused, and the patient left the hospital March 4, 1936.

June 9, 1937, she was readmitted with a history of amenorrhea of six months' standing, enlargement of the pelvic mass to reach the umbilicus, a prolonged fever, and marked anemia. Operation was performed June 21. A large tumor, suggesting fibromyoma or possibly malignant ovarian tumor, occupied the lower part of the abdomen and seemed frozen to the pelvis. The mass was friable, and the intestine and omentum were congested and adherent to the uterus and to the anterior abdominal wall. The postoperative wound discharged a large amount of foul-smelling yellowish green material, from which Bacterium coli was recovered. Biopsy showed a granulomatous reaction of an unusual type. The patient died August 12, almost two months after operation.

*Necropsy.*—The right ovary was replaced by a spherical, spongelike mass, from which exuded greenish white cheesy purulent material. This mass measured 10 by 10 cm. From the honeycomb-like substance many pinhead-sized white nodules could be expressed. On the uterine surface to which the mass was attached, the fallopian tube and a solid portion of the mass were coated with black tissue, all embedded in adhesions. The peritoneum, omentum and intestine were flecked with pus. The omentum was densely adherent to the mass and partially necrotic. A portion of sigmoid adherent to the left and posterior surfaces of the mass was the seat of a perforation, from which exuded fecal matter and purulent material along a sinus tract to the abdominal wound. The liver contained a solitary metastatic nodule, about 3 cm. in diameter. There was a deep-lying psoas abscess low down under the left inguinal ligament, which did not involve the hip joint, lumbar muscles or vertebrae.

Microscopic sections of the ovarian mass showed the typical sponge or honeycomb appearance of loose granulation tissue with numerous small abscess areas containing granules typical of the colonies of the ray fungus. The metastasis to the liver also showed colonies of actinomycetes, with purulent necrosis, surrounded by granulation tissue.

DISCUSSION

SYLVAN E. MOOLTEN: It might be worth while to emphasize the statement of Dr. Auster that dissemination of actinomycetes is through local extension or by way of the blood stream and not through the lymph stream. I saw recently at St. Peter's Hospital, in New Brunswick, a young woman of 27 years with actinomycosis of both ovaries, apparently of hematogenous origin. An appendectomy had been performed a year prior to this, and since that time she had been having symptoms of bilateral pelvic inflammatory disease and a more or less continuous septic fever, with development of marked anemia. She had been examined by several consultants, all of whom believed her condition was of gonococcic origin, despite the absence of gonococci from cultures and smears. Finally, two weeks ago, she was operated on and the adnexae uteri were removed from both sides. These showed cystic enlargement of the ovaries, the right being about the size of an orange, and the left about half that size; both were considerably inflamed. The left ovary presented a partially healed granulomatous condition with little pus. Yellowish areas were found which microscopically showed groups of foam cells. The right ovary was honeycombed with pus, was grayish and rather foul smelling, and had abundant shaggy exudate lining the pus pockets, as well as some granulation tissue. Histologic examination revealed the ray fungus (photographs were shown) scattered within the purulent exudate, which was chiefly polymorphonuclear leukocytes. The tube on the right side showed slight chronic inflammation of a granulomatous type with no particular evidence of active disease. On review of the sections of the appendix, taken out a year ago, the original diagnosis of mild catarrh and subacute periappendicitis was confirmed, the periappendical lesion being chiefly of a mild granulomatous nature, with

plasma cells and fibroblasts, and apparently of extrinsic origin. In reviewing the case I felt it proper to conclude that both ovaries had been infected primarily, so to speak, and independently of each other, probably from the blood stream, and that the lesion in each had progressed at a different rate and with a different and independent course. The fallopian tubes and appendix were involved only by continuity. Neither the primary focus nor the portal of entry could be found, but her teeth are under suspicion because of their poor condition. At the present time she is doing well.

PAPILLOMA CHOROIDEUM OF THE FOURTH VENTRICLE. ANDREA SACCONE AND ABRAHAM ROSENTHAL.

This paper will be published in full in the ARCHIVES OF PATHOLOGY.

DISCUSSION

AMOUR F. LIBER: I should like to ask whether there was a cone of pressure in the tonsils of the cerebellum. I could not make out whether there was from the lantern slide shown, because it showed the floor of the fourth ventricle instead of the ventral aspect of the cerebellum. I think this matter is important because, from the clinical course outlined, the spinal fluid was tapped, and somewhat later the patient died, with some evidence of involvement of the medulla oblongata. I think it cannot be repeated too often that tapping the spinal fluid is dangerous if there has been long-continued intracranial hypertension, particularly when a tumor is in the posterior fossa. One of the purposes of pathologic investigation is to point out to clinicians their mistakes, and this is a case in point; at least, I think it is. I should like to ask secondly whether in the stroma of the papillae in the tumor there were large and rather coarse elastic fibers such as are seen in the normal stroma of the choroid plexus—the non-neoplastic choroid plexus.

ANDREA SACCONE: The spinal fluid was tapped several times, and nothing happened to the patient. In this case I think the fluid was tapped when the tumor was not completely obliterating the fourth ventricle.

In reply to the other question—with all the different stains used we found coarse elastic fibers without any appearance of mucin, and that was one of the reasons for concluding that it was a tumor arising from the choroidal epithelium and not from the ependyma.

CYSTIC HYDROPS OF THE PINEAL GLAND. AMOUR F. LIBER.

Three kinds of cysts occur in pineal glands. A small cavity which does not cause enlargement of the gland occurs in about 30 per cent of adult pineal glands. A second variety of cyst is associated with teratoma or pinealoma. A third variety is a large cyst which distends the gland and which may compress neighboring organs, such as the brain stem and the veins of Galen. A condition of the last type, termed cystic hydrops by Virchow, is apparently rare, and few cases have been reported. The present case concerns a 43 year old white woman who died with lobar pneumonia, septicemia due to an infection with the hemolytic streptococcus, and meningitis. The pineal gland was distended by a single cyst measuring 14 by 10 by 9 mm. The wall was very thin and made up of a layer of pineal tissue lined rostrally by glia and caudally by a single layer of parenchymal cells on a thin collagenous basement membrane. The blood vessels in the gland presented no alterations, and there was no evidence of ischemic changes. The fluid in the cyst was coagulated by solution of formaldehyde U. S. P. It contained a few fresh red blood cells, but no fibrin, no free hemoglobin and no mucin. The glial lining of the cavity was continuous rostrad with a dense glial mass, which pushed apart the habenular and posterior white commissures, filling in the pineal recess and bulging slightly beneath the ependyma. The quadrigeminal plate was markedly flattened, but the aqueduct of Sylvius was not narrowed. The mesoceliac recess was reduced to a narrow cleft. The veins of Galen were

not compressed. The cavum septi lucidi was markedly dilated, extending uninterruptedly from the genu to the splenium of the corpus callosum. There was no internal hydrocephalus. The meningitis was not associated with any gross or microscopic lesion of the fixed tissues in the brain or of the meninges or of the bones of the skull. The central situation of the cyst and the presence of a glial investment continuous with a glial mass obliterating the pineal recess of the third ventricle are arguments in favor of Krabbe's theory that cyst of the pineal gland originates from a glial tractus diverticularis filling the erstwhile pineal diverticulum. The accumulation of fluid in, and the enlargement of, the pineal gland have received no satisfactory explanation.

THE PHARYNGEAL PITUITARY GLAND. R. H. MELCHIONNA (by invitation).

Fifty-four unselected cases of pharyngeal pituitary gland were studied. The material was obtained at autopsies. After fixation of the specimen in solution of formaldehyde U. S. P., a midline block of the nasopharyngeal mucosa was carefully stripped from the vomer and sphenoid bone in the vicinity of the articulation, embedded in paraffin, step-sectioned and stained. The pharyngeal pituitary gland was located microscopically in 51 of the 54 specimens. For comparison in each instance, a study of the pituitary gland was included.

The pharyngeal pituitary gland is most constantly located, as a small piece of typical or atypical pituitary tissue, in the midline, deep in the mucosa, contiguous to the periosteum, at a point where the periosteum is firmly attached to the vomer-sphenoid articulation. While it is frequently found as a well circumscribed or well encapsulated area, it often shows irregular cords extending out from the main mass of cells in various directions. The largest gland observed in this series measured 6.62 by 1.15 by 0.35 mm.; the smallest, 0.36 by 0.1 by 0.21 mm. From an analysis of the variance of the sum of the three dimensions of the pharyngeal pituitary gland and age by decades, it appears that there is no progressive evolution of this structure during life.

The pharyngeal pituitary gland is composed essentially of two types of cells: (1) epithelium and (2) differentiated cells similar to those in the anterior lobe of the pituitary gland. Epithelium was present in 33 of the 51 glands examined. It was usually of the transitional type, arranged in small nests with an indefinite basal layer. An evaluation of the relative amount of epithelium in each gland in relation to age indicates that there is no progressive differentiation of the cells, such as is found in other functional organs.

The differentiated tissue in the pharyngeal pituitary gland has the same microscopic appearance as that in the pituitary gland, but there are conspicuous quantitative differences. As regards the acidophils, with the exception of 6 glands there was a deficiency of basophilic and acidophilic cells. In 17 and 18 of the glands the acidophils and basophils, respectively, were entirely absent. Even when these cells were present, the relative number was small, usually less than 1 per cent.

The importance of accessory hypophysial tissue in the explanation of discrepancies in observations on both experimental animals and man is apparent. In this connection, a number of cases in which the pituitary gland showed pathologic changes are of especial interest, although not particularly enlightening.

It is concluded that under normal conditions of growth and activity it is unlikely that the pharyngeal pituitary gland contributes any significant physiologic function, but in some cases of an alteration in the activity or in the structure of the pituitary gland it cannot be denied that the pharyngeal pituitary gland may undergo structural alteration and serve as an endocrine organ.

DISCUSSION

SIMON L. RUSKIN (by invitation): I hope you will pardon me as a rhinologist coming into a meeting of pathologists, but this exceptionally important paper is of great interest to me since I have in press a manuscript pointing out, I believe for the first time, a disease of the pharyngeal pituitary gland, and I have to take

issue with Dr. Melchionna when he states that knowledge of the pharyngeal pituitary gland has no clinical value. I think it has immense clinical value. I spent over four years following the pathologic changes and the clinical manifestations and have been able to correlate disease of the pharyngeal pituitary gland with an old described disease, mention of which disappeared almost entirely from the literature for a period of almost forty years, known as Thornwaldt's disease. The original publication concerning it appeared in 1889 and was popularly discussed for years; then interest in the subject disappeared, until within the last few years, when occasional reports have been made. I feel that Thornwaldt's disease is not a disease of the medial recess of the nasopharynx and that it has nothing to do with Luschka's pharyngeal bursa, as Thornwaldt believed, because the deep cherry red spot which he described as seen between the alae of the vomer is not a simple erosion but is exposed pharyngeal pituitary gland. That cherry red spot was never studied pathologically, and was not followed in his time. However, that cherry red spot is associated with a definite clinical syndrome: pain in the back of the head, pain in the root of the nose and a remarkable vasomotor sensitivity to changes in temperature. The patient, in addition, suffers as a rule from low grade anemia, which is difficult to control. Usually there is a history of a streptococcic infection, a severe streptococcic sore throat. Whether the streptococcic infection settled in that particular area, inflamed the pharyngeal pituitary gland and eroded and exposed it, I do not know, but patients with this disease have a remarkable degree of anemia, which is resistant to most forms of therapy. They are sensitive to heat and cold and have headaches, simulating sinusitis.

Frequently there are associated cysts. These are seen better in the lower animals; they have been described in dogs. I have sectioned a series of sheep and have 500 serial sections in which I was able to show considerable pharyngeal pituitary tissue, apparently a much larger amount than is observed in man. I went so far as to make an extract of this particular section of the sheep's nasopharynx, injected it into immature white mice and observed a swelling of the uterus of the immature white mouse and what looked like graafian follicles. When I sent these results to the editors of a journal of rhinolaryngology about four years ago they sent the paper back to me with a request for further substantiation of the possibility of an extract of the pharyngeal pituitary. The extract used probably contained hormones, a matter which should be controlled by clinical observation. I think it will be found that recognition of pharyngeal pituitary disease will play a role particularly in the diagnosis of those obscure conditions which ordinarily are studied by exploration of sinuses instead of by this particular study. I think my work may be considered as showing the first recognition of pharyngeal pituitary disease on record.

PAUL KLEMPERER: I listened to this paper with interest because I remember the time when Erdheim and Haberfeld established the anatomy of the pharyngeal pituitary gland. In the years since then this gland has been almost forgotten. Few reports appeared later, and, if I am not mistaken, this is the first time in this country that the pharyngeal pituitary gland has been studied in such an exhaustive way. It is rather surprising that the very active laryngologists and rhinologists of this country have not discovered it and removed it, and I am glad to hear that Dr. Ruskin has considered doing this. However, I should like to ask him: Were these cherry red granules actually pituitary tissue? Were they removed and sectioned?

SIMON L. RUSKIN: No, they were not removed, but examination of the tissue in a cadaver will corroborate the statement that a definite change in color occurs between the small area where pharyngeal pituitary tissue is excised and the surrounding tissue; however, the removal of this pharyngeal pituitary tissue from the living patient is difficult; it bleeds freely. I might add one other point: that this is a frequent source of idiopathic postnasal bleeding. Patients who have nasal bleeding that does not occur from the septum but from the nasopharynx have it from this location.

ROBERT A. MOORE: I might emphasize that one pathologist does not have an opportunity to see many cases in which there is destruction of the pituitary gland, perhaps by a cyst, perhaps by a metastatic tumor or even by a tumor of the pituitary gland itself, as in cases of acromegaly. This piece of pharyngeal tissue may be removed easily with a chisel and saw, and apparently there is no leakage from the undertaker's injections. The preparation of the sections is not too laborious in a single case.

R. H. MELCHIONNA: In answer to Dr. Ruskin's question as to whether or not my co-workers and I have seen small red areas representing the pharyngeal pituitary gland in the nasopharyngeal mucosa of the cadaver, we have not identified the pharyngeal pituitary gland grossly. We have been unsuccessful in attempting to locate this structure by gross inspection or by the aid of the dissecting microscope on the mucosal surface, the cut periosteal surface or in midline saggital sections. The pharyngeal pituitary gland is frequently large enough to be plainly visible in stained paraffin sections, but apparently there are no features that distinguish it in fresh or formaldehyde-fixed material.

In the human being, the pharyngeal pituitary gland is located just anterior to the adenoid, deep in the mucosa. With enlargement through inflammation, the adenoid occasionally overlaps the pharyngeal pituitary gland, which is presumably fixed in position. The pharyngeal pituitary gland was not found in 3 cases. Whether adenoidectomy had been performed in these cases we have not been able to learn definitely from hospital records. Adenoid tissue was present histologically. The pharyngeal pituitary gland, being deeply located in a small recess at the vomer-sphenoid articulation, would probably not be removed by the ordinary methods used in adenoidectomy.

The minute size and the difficulty of gross recognition of the gland are barriers to assays of the function of this gland in man. It is interesting to know of Dr. Ruskin's study in sheep, and the assays if cautiously controlled are enlightening. Ovarian stimulation by injection of extracts from the pharyngeal pituitary gland in carefully controlled experiments is probably indicative of function. I was not aware of the presence of the pharyngeal pituitary gland in sheep, although the gland is known to occur in the chimpanzee and in the cat.

PATHOLOGIC CHANGES IN THE HEART IN SUDDEN DEATH. JAMES R. LISA.

A review of 41 cases of sudden death due to cardiac disease is presented. The patients included men and women, white and colored. The ages ranged from 1½ months to 75 years.

In 2 cases there were no demonstrable changes in the heart sufficient to explain the cardiac type of death. Of the other 39 cases, chronic arterial disease played the predominant role in only 6, all the patients in these instances having marked arteriosclerosis and acute coronary thrombosis.

In 14 cases an infectious lesion directly affected the heart; in 4 of these there was acute rheumatic myocarditis; in 5 acute infectious endocarditis; in 2 acquired syphilis; in 2 combined syphilis and essential hypertension with a superimposed acute infectious endocarditis; in 1 acute coronary insufficiency with bacterial arteriolar emboli and miliary infarctions.

In 12 cases there was acute toxic myocarditis associated with acute pulmonic infection; in 2 of these cases there were combined syphilis and rheumatic heart disease; in 2 there was acute coronary thrombosis; in 3 acute coronary insufficiency, associated in 2 of the cases with acute medial necrosis of the coronary arteries; in 2 pneumonia. Three of these cases occurred in infancy and childhood.

In 6 cases there were acute myocardial lesions, presumably toxic, associated foci of active infection; in 2 of these cases, there was syphilis combined with essential hypertension, in 2 acute coronary thrombosis; in 1 acute coronary insufficiency. One case in the latter group occurred in childhood. In a case in infancy in which similar myocardial lesions were observed, no toxic or infectious factor was demonstrable.

An anatomic basis for sudden heart block and ventricular fibrillation is suggested.

DISCUSSION

HARRY VESELL: I should like to ask whether Dr. Lisa has not been able to find many, if not most, of these histologic lesions in patients who die a much more gradual death, that is, who do not die within a few minutes.

JAMES R. LISA: Lesions of this character are present in cases of more gradual death. There is not much difference in the pathologic changes of this group and a group in which death occurs in from one or two to six hours; in fact, the changes are the same. The one striking thing about this series is the extent of the lesions. They are much more extensive, although in character they are exactly the same.

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## CHICAGO PATHOLOGICAL SOCIETY

FRANCIS D. GUNN, *President*

*Regular Monthly Meeting, Jan. 10, 1938*

EDWIN F. HIRSCH, *Secretary*

TOXIC CHANGES IN THE SPINAL CORD RESULTING FROM SPINAL ANESTHESIA. WILLIAM H. SWEET, HUBERTA LIVINGSTONE and GERALDINE LIGHT.

The toxic action in the spinal cord of the agents used for spinal anesthesia has been investigated in animals since the first decade of the present century. It has been shown that the common spinal anesthetic agents, procaine, tropacocaine and stovaine, cause in animals severe degeneration of cells in the anterior horn, peripheral demyelinization around the entire circumference of the cord, with swelling and fragmentation of axons in the involved areas, and patchy demyelinization of fibers in the anterior and posterior roots.

We found two reports of delayed death following spinal anesthesia, with clinical symptoms of an involvement of the cord in which complete studies were made post mortem. Two cases have been reported, one by Nonne and Demme abroad and the other by Brock, Bell and Davison in New York City, in which following anesthesia obtained with tutocaine and nupercaine, respectively, the spinal cord presented changes similar to those described in animals. We now report a third case.

A woman aged 38 years, who was in a state of severe cachexia, had a portion of the ileum resected for terminal ileitis under spinal anesthesia induced with 140 mg. of procaine crystals. On the tenth postoperative day, incontinence of the bowel and bladder developed. On the eighteenth day she was dyspneic and the next day a diagnosis of paralysis of the diaphragm was made and confirmed by fluoroscopic examination. At this time she had flaccid paralysis of both lower extremities and a Babinski sign on each side. She died on the twenty-eighth postoperative day.

In tissues outside the central nervous system were numerous abscesses. The brain had several pinpoint hemorrhages in the right mamillary body. There was a zone of demyelinization around the entire periphery of the sacral, lumbar and thoracic sections of the cord and to a less extent around the cervical portion of the cord, and in the medulla and pons the peripheral demyelinized zone was again striking. The nerve roots had severe demyelinization throughout the length of the cord. Among the ganglion cells of the cord in the sacral, lumbar and thoracic regions, there were some with severe retrograde degeneration. Since these changes correspond with those seen in experimental animals and in patients following spinal anesthesia, we conclude that they were caused by the procaine, although the patient's marked debility probably reduced the resistance of the spinal cord to the toxic action of the anesthetic.

DISCUSSION

ARTHUR WEIL: I can add another case, in which an operation in the lower part of the abdomen was done under spinal anesthesia and about two weeks later a second operation. The patient died about three weeks after the first operation. There was marked edema of the periphery of the spinal cord in the lower dorsal and lumbar regions. The Marchi preparations demonstrated marked degeneration of the myelinated fibers in the posterior roots. There were no cellular exudates in the meninges. The changes observed in the cord by Dr. Sweet might be ascribed to toxic conditions associated with the chronic illness of the patient. However, all the reports on changes in the spinal cord following spinal anesthesia, with peripheral distribution of the degeneration, agree with Dr. Sweet's description. I did not succeed in producing degeneration of the rat's spinal cord in test tube experiments with certain spinal anesthetics. Such degeneration was produced in fifteen hours with weak solutions of derivatives of barbituric acid, such as 0.5 per cent nembutal (pentobarbital sodium).

INFARCTION OF BONE. D. B. PHEMISTER.

Four cases of multiple massive aseptic necrosis of bone are reported, in three of which the lesions were definitely and in one presumably due to caisson disease.

CASE 1.—A 61 year old man had severe caisson disease 36 years before death from carcinoma of the lung. The disease produced pains in the lower limbs and gradually increasing pains and stiffness in the hips, so that for twenty years he had been severely incapacitated. Roentgenograms taken three years and again shortly before death showed marked collapse of the heads of the femurs and deforming arthritis of the hips, and areas in the lower 6 inches (15 cm.) of the interior of the shaft of each femur and in the upper 5 inches (12.5 cm.) of the left humerus demarcated by an uneven zone of increased density. At autopsy these demarcated areas were shown to be infarcts with calcification and partial ossification of the surrounding zone of demarcation. The heads of the femurs were collapsed; the articular cartilages were destroyed and the bone at the articular margins markedly overgrown. The changes were similar to those seen long after fracture of the femoral neck with death of the head, union of the fracture, subsequent collapse of the head, partial or complete replacement of the dead bone by new bone and deforming arthritis of the hip joint.

CASE 2.—A 37 year old man had severe caisson disease five years before examination, resulting in pains and gradually increasing limitation of motion in both hips and both shoulders. Roentgenograms showed partial collapse, greater density and incomplete sequestration of the major portion of the head of each femur and similar but much less marked changes in the head of each humerus. Biopsy showed the lesion of the head of the right femur to consist of aseptically necrotic bone.

CASE 3.—A 54 year old man had caisson disease twenty-nine years before examination. It resulted in permanent spastic paraplegia of the lower extremities, which interfered markedly with walking, but there was no history of pains in the bones. Roentgenograms of the skeleton showed regions in the lower 5 inches of the shafts of the femurs and in the upper and lower ends of the shafts of the tibias and fibulas quite similar in outline and density to those seen in case 1. No biopsy was made.

CASE 4.—A man 50 years old gave a history of an attack of "severe rheumatism" occurring nineteen years previously, which left him with a painful swelling of the left knee that had gradually grown worse. His occupation was given as that of a railroad construction worker, but a history of work in compressed air was not investigated. Roentgenograms of the skeleton revealed regions in the lower 4 inches (10 cm.) of the shafts of the femurs and in the upper and lower 3 to 4 inches (7.5 to 10 cm.) of the shafts of the tibias similar in outline and density to those in cases 1 and 3. Death resulted from cerebral hemorrhage. At

autopsy there was marked villous arthritis of the left knee. Examination of the excised lower third of the left femur revealed a walled-off and partly calcified necrotic region comprising the lower 3 inches of the interior of the shaft and parts of the condyles of the left femur, quite similar to the lesions in case 1. Because of the identical pathologic changes, the presumption is that the bone and joint changes were results of caisson disease.

The method of production of the bone lesions appears to be as follows: While in the caisson, excess nitrogen is absorbed by the blood which in turn is taken up by the tissues, in largest amounts by fat and lipoids. The marrow of the bones in the extremities being rich in fat, absorbs much of the nitrogen. When decompression occurs too rapidly, nitrogen is liberated in bubbles within the bones, which may result in compression and necrosis of medullary and cancellous tissues. Embolism of the arteries of the interior of the bones appears to be a much less probable explanation. The accompanying villous and deforming arthritis is a change secondary to the necrosis of bone bordering on the joint.

DISCUSSION

EDWIN F. HIRSCH: The occurrence of such extensive aseptic necrosis of bones in caisson disease establishes another causal agent of bone necrosis.

PETER BASSOE: In 1910 the Illinois state legislature provided a grant for the study of occupational diseases. Under this Dr. Alice Hamilton made a notable study of metallic poisons. The study of caisson disease was assigned to me. I examined from 200 to 300 of these so-called sand hogs. Most of them had worked under compressed air in water tunnels and in submerged caissons in bridge construction. Clinical information was difficult to obtain because many were inebriates. Interpretation of the physical changes was also vague because so little was known of the disease. The escape of nitrogen into the tissues was understood. I attempted to differentiate the joint changes of arthritis deformans and the arthropathies due to lesions of the spinal cord, such as tabes and syringomyelia. Dr. Phemister's report has clarified knowledge of many of the lesions in bones not understood at that time.

I. DAVIDSOHN: With so much destruction of the bone marrow, were there changes in the blood, spleen or other viscera?

D. B. PHEMISTER: Only varices of the leg were noted in one patient.

GROSS CONGENITAL MALFORMATION OF THE SPINAL CORD IN A CHILD. PAUL C. BUCY.

A 4½ year old girl was referred from the Children's Memorial Hospital. She entered the University of Chicago Clinics on Nov. 27, 1937. Her birth and early development had been normal. She had complained occasionally of pain and stiffness of the neck, but these attacks were short and attracted little attention. On September 16, she awakened during the night with pain and stiffness of the neck. A diagnosis of acute anterior poliomyelitis was made (an epidemic of this disease was present), and she was admitted to the Municipal Contagious Disease Hospital, where the diagnosis was not confirmed.

Early in October, because of similar complaints, she was again admitted to the same hospital, where a diagnosis of congenital deformity of the neck was made. She was then transferred to the Children's Memorial Hospital, where examination revealed the neck to be stiff and movements painful. The chin was rotated to the right. There was marked weakness of both arms; all tendon reflexes were absent; Babinski's sign was not present; sensation was diminished below the neck. The child grew worse steadily and on November 27 was transferred to the University of Chicago Clinics. At that time, she was anesthetic below the chin; there was complete flaccid paralysis of both arms and both legs and of all intercostal muscles; marked flexor defense reflexes were present; all tendon reflexes and the abdominal reflexes were absent. Babinski's sign was present bilaterally. She was incontinent of urine and feces.

Lumbar puncture showed normal spinal fluid and no block. Roentgenograms revealed marked dilatation of the cervical and upper thoracic portions of the spinal canal and a spina bifida of most of the cervical vertebrae. These facts were confirmed at operation. A diagnosis of congenital tumor of the spinal cord was made. A laminectomy from the first cervical to the fifth thoracic vertebra was done on December 2. The patient died on December 3 of respiratory paralysis.

The operation and necropsy revealed a marked and unusual congenital malformation of the spinal cord. Beneath the dura was a large cyst enclosed by an arachnoid-like membrane. This cyst was separate from the subarachnoid space. From the level of the first cervical to the fifth cervical vertebra the cyst lay posterior to the spinal cord, which was flattened and formed its anterior wall. On the dorsal surface of the spinal cord at this level were many brown papillomatous structures, grossly considered to be masses of choroid plexus. At the level of the sixth cervical vertebra the dorsal surface of the spinal cord split, leaving a large opening through which the cystic cavity passed. At this level the spinal cord consisted of two large lateral masses which surrounded the cystic cavity and were united anteriorly by a thin sheet of tissue. At about the level of the fourth thoracic vertebra the two halves of the spinal cord united again, but there was a hydromyelic cavity in the center of the cord.

This malformation undoubtedly represents a defective closure of the neural tube in the lower cervical region associated with a spina bifida of the vertebrae.

FUSOSPIROCHETAL INFECTION FROM THE BITE OF AN ORANG-UTAN. I. PILOT.

An attendant at a zoological garden was bitten through the palm and dorsum of the right hand by an orang-utan, which left a deep irregular wound and fractures of the upper ends of the second and third metacarpal bones. Infection developed rapidly despite prompt first aid cleansing. There were marked swelling of the dorsum of the hand and fever. A fetid exudate oozed from the wound, and the hand and wrist became swollen. Surgical incisions exposed deep necrosis of the tissues. Stained preparations of the exudate revealed numerous fusiform bacilli and spirochetes corresponding to the Vincent type of organisms found about the teeth and tonsils. Cultures on blood agar yielded many hemolytic streptococci of the type beta. These, by the Lancefield method of grouping, belonged to group A, or the human type. After several months of surgical treatment and injections of neoarsphenamine the patient recovered from the infection, but the hand was considerably stiffened and deformed.

DISCUSSION

E. PIETTE: An attendant at the Brookfield Zoo had a gas bacillus infection of the arm which developed about a week after he had been bitten by a chimpanzee.

EDWIN F. HIRSCH: During the World's Fair in 1934 an animal trainer was bitten on the arm by a lion. An organism with the cultural characteristics of Bacillus mallei was recovered from the wound.

FRANCIS D. GUNN: Do spirochetal organisms alone produce the lesion or must they be associated with some other organisms?

I. DAVIDSOHN: Three different conditions of the blood have been ascribed to spirochetal infections: (1) a leukemoid state with the leukocyte count ranging up to 60,000 per cubic millimeter and showing a shift to the left, (2) agranulocytosis and (3) mononucleosis, ascribed to a systemic infection by these organisms. What changes, if any, did you observe in your patient?

I. PILOT: An injection of spirochetal organisms alone does not produce a lesion. The opinion is that first a pyogenic organism enters the tissues and later the spirochete. All the patients observed showed a response of the polymorphonuclear leukocytes and not of the mononuclear leukocytes. As a rule, spirochetal infections are associated with lowered resistance and marked alterations in the composition of the blood in various disorders.

# Book Reviews

**Pathology of the Central Nervous System. A Study Based Upon a Survey of Lesions Found in a Series of Fifteen Thousand Autopsies.** By Cyril B. Courville, M.D., Professor of Neurology and Psychiatry, College of Medical Evangelists, and Director, Cajal Laboratory of Neuropathology, Los Angeles County Hospital, Los Angeles, Calif. Cloth. Pp. 344, with 200 illustrations. Price, $5.75. Mountain View, Calif.: Pacific Press Publishing Association, 1937.

There is a distinct need for a thorough-going encyclopedic text of neuropathology written in English. The present monograph, in the excellence of its typography and illustrations, gave rise to the hope that it in some measure would fill that need. It does not. There now exist some four texts, recently written, by both American and British authors. Each is superior to this one.

The correlation of clinical neurology and neuropathology, which the author has attempted but poorly attained, desirable as it is, is beyond the scope of any monograph on neuropathology and can find adequate expression only in a complete text on clinical neurology. That the author has been interested chiefly in the effect of trauma on, and neoplasms of, the central nervous system is obvious. To these subjects he devotes, respectively, 60 and 56 pages of the total of 344, while epidemic encephalitis is disposed of in 1 page (the St. Louis type receives a single sentence, in a footnote, and the Japanese variety is not mentioned). Two scant pages are given to multiple sclerosis; three lines to lead encephalopathy, four lines to Huntington's chorea and a sentence to Wilson's hepatolenticular degeneration. Throughout the entire text the reader is appalled at the manner in which microscopic studies, even those on subjects on which the author has written extensively elsewhere, are virtually completely ignored. In addition to these omissions and inadequacies, numerous errors are introduced either directly or by inference. The typographic errors, of which there are very few, do not concern us. Permit a few of the misstatements to be cited:

On page 31 it is stated that the "sucker feet" pass *through* the perivascular space to attach to the blood vessels. On page 62 it is stated that *"Congenital* hydrocephalus may result from a surgical attempt at correction of cranial or spinal meningoceles." On page 90 the author indicates that sinus pericranii is a dilatation of the diploic veins. On page 95 he implies that only angioma in the central region of the cerebrum is associated with convulsions, whereas such seizures are common with angioma in any part of the cerebral hemispheres. On page 143 he states that gumma of the dura "occurs in the form either of a flattened plaque or of multiple confluent *tubercles."* On page 150 the only discussion of the microscopic appearance of a gumma of the central nervous system consists solely of the statement "microscopically it resembles a tuberculoma." On page 155 he makes the statement that the "symptoms [of tabes dorsalis] are confined as a rule to the lower extremities, at least in the early stage of the disease," thus ignoring the pupillary changes, the sensory changes over the face and chest and the gastric crises. On page 156 the blood vessels in tabes dorsalis are described as showing the "characteristic alterations." On page 176 the author gives "posterior poliomyelitis" as synonymous with "herpes zoster" and then goes on to state that the pathologic changes are confined to the ganglion of the affected sensory nerve. In the discussion of birth injuries (pages 182 to 190) the impression is clearly given that subdural hematoma in infants, unlike that in adults, resolves and does not go on to the formation of large encapsulated cysts which give rise to clinical manifestations. Such results from subdural hematoma are, of course, common in children. On page 198 one finds a section headed "The effect of repeated blows to the head ('punch drunk')" and nothing in the section relative to the morbid

anatomy of that condition. The discussion of the progressive muscular atrophies is quite misleading. On page 295 there occurs the distinct implication that localizing signs are frequently absent in cases of cerebellar astrocytoma. On page 299 the description of medulloblastoma as having a "fine reticular stroma of connective tissue" is wrong and applies instead to the not infrequent primary sarcoma of the meninges which occurs in this region and which may be confused with the medulloblastoma. On page 300 the statements that astroblastoma does not show extensive intervascular necrosis and that the intercellular material in the oligodendroglioma is made up in part by strands of connective tissue are both erroneous. This list makes no pretense of being all inclusive.

T. C. BUCY.

**The Histopathology of Malaria with Special Reference to the Function and Origin of the Macrophages in Defence.** By William H. Taliaferro, professor of Parasitology, University of Chicago, U. S. A., and H. W. Mulligan, of the Malaria Survey of India, at Kasauli, under the Indian Research Fund Association. Memoir no. 29. Paper. Pp. 138, with 15 plates. Price, rupees 3-8-0 or 5 shillings. Calcutta: Thacker, Spink & Co., Ltd., 1933.

This monograph is based on researches by the authors on malaria in monkeys infected with the Oriental species of plasmodia at the laboratories of the Malaria Survey of India, in Kasauli, India, conducted under the auspices of the Indian Research Fund Association. The monograph consists of three parts: Part 1 includes a general discussion of the histology of malaria with special reference to the function and origin of the macrophages in defence; part 2 describes the cellular reactions in Silenus rhesus during the course of infection with Plasmodium cynomolgi and with Plasmodium knowlesi. Tissues from seventy specimens of Silenus rhesus were studied. Twelve of the animals were normal; forty-three were experimentally infected with P. knowlesi, of which twelve were superinfected with various strains of P. knowlesi; fourteen were infected with P. cynomolgi and one with both P. knowlesi and P. cynomolgi. In addition, nine monkeys (Silenus irus) were studied, four apparently normal, two infected in nature and three experimentally infected with P. knowlesi. Part 3 is a bibliography of 240 references.

Part 1 includes a thorough review of the literature on the pathology of malaria with the object of attempting "to present a critical review of the literature on the pathological changes observed in malaria, chiefly in the human disease, and to emphasize those aspects of the subject which relate to the defence of the body against malaria rather than those which involve general pathological processes." In this review, special attention has been paid to the question of "the origin, function and developmental potencies of the cells of the connective tissue and blood which are chiefly concerned in malarial defence." There is also a review "arranged according to the organs involved, of the general pathological changes observed in malaria which are of importance in the cellular phases of the defence reaction," and "a general consideration of immunity to malaria with special reference to the functions of the phagocytic cells." The authors point out the close resemblance which exists between avian, simian and human malaria and the value of such material in furnishing information regarding "the source and cytogenesis of the newly-formed macrophages which are associated with the defence of the body against malaria."

Space will not allow a complete review of the conclusions of part 1, but this much may be said: "The histologic studies of this and other experimental material tends to confirm the principal ideas of Maximow in regard to the genesis of the cells of the connective tissue and blood. The authors suggest the term "lymphoid-macrophage system" for the reticulo-endothelial system "in order to include both the macrophages and their precursors, all of which are involved in malarial and other defence reactions." They present much detailed evidence pointing to the idea that the general defense reactions of malaria are essentially "local reactions in strategically placed organs" and that immunity to malaria rests essentially on

a cellular basis of stimulation of the lymphoid tissues with consequent building up of a mesenchymal reserve from which macrophages are formed through the transformation of lymphocytes and monocytes into macrophages.

These ideas are elaborated in part 2, based on the experimental findings. The final conclusion in regard to the defense against malarial infection is that "defence against malaria, as evidenced by the death of parasites during the acute rise of cynomolgi or of knowlesi infections (natural immunity) or by the increased death and destruction of parasites during and after the crisis of P. cynomolgi infections (acquired immunity), is essentially a local immunity in strategically placed organs. Phagocytic activity, lymphoid hyperplasia, and the concomitant cytogenesis of macrophages are initiated in the spleen and are always most pronounced in this organ. As the intensity of malarial stimulation increases these changes also occur, though to a less degree, in the liver, and to an even less extent in the bone marrow."

Fifteen superb plates present photomicrographs illustrating the histologic and cytologic changes observed. Many of these are detailed drawings by Miss Esther Bohlman, illustrating cytologic details of cellular response.

This monograph will be of great interest and value to all pathologists interested in the pathologic and clinical features of both human and experimental malaria, as well as to others interested in the general problem of morphogenesis of the blood and connective tissues and its relationship to the general defensive mechanisms against infectious disease.

**Les immunites locales.** By A. Besredka, professeur a l'Institut Pasteur. Paper. Pp. 224. Price, 35 francs. Paris: Masson & Cie, 1937.

This monograph is a recapitulation and extension of the monographs which Besredka has been publishing on this subject for the past thirty years or more. (The last monograph that was translated into English was published in 1927 by the Williams & Wilkins Company, Baltimore.) Briefly stated, Besredka believes that certain tissues have cells receptive to certain bacteria. Thus in immunization the antigen should be directed to the tissues by which the invading organism enters or infects. He discusses in separate chapters the diseases of the skin, mucous membranes of the mouth, pleura, lungs, intestines and peritoneum. He cites the diseases most commonly affecting each of these sites and gives experimental and clinical evidence to show that immunization of the tissue representing the portal of entry is the most effective. For immunization he uses either a vaccine (heat killed) or what he calls an "antivirus," which is simply a filtrate of a broth culture of the bacteria in question. These products of the bacteria supposedly act directly against the bacteria, and thus aid the receptive cells of the tissues. The "antivirus" or the vaccine is best applied superficially and, depending on the organ, by moist dressings, sprays or ingestion. The mechanism of this type of immunization is local, as no humoral antibodies are demonstrable. Immunity is said to be manifest a few hours after the application.

Thousands of persons have been subjected to oral vaccination against typhoid in France and its colonies as well as in Japan with apparently good results. However, bile must be administered with the heat-killed organisms to ensure that the mucous membranes will be sufficiently irritated to allow the passage of the vaccine into the wall of the intestine. In such cases agglutinins appear in the blood. Unquestionably, the oral method of vaccination would be preferable to the subcutaneous if the former were as efficacious as the latter, but not enough evidence is presented to prove this point.

The chapter on immunization of plants is interesting and strengthens the hypothesis that immunity is local, accomplished by the tissues, as plants have no circulation like that of animals.

Of more recent date is the last chapter, on local passive immunity, in which Besredka cites evidence in animals to the effect that the application of antiserum should also be directed locally to the site of entrance of the infecting organism.

Sheep and goats, for example, were protected from many fatal doses of tetanus toxin if a salve of antitetanic serum had been applied an hour or more before to the site of inoculation.

**Genital Abnormalities, Hermaphroditism and Related Adrenal Diseases.** By Hugh Hampton Young, M.A., M.D., Sc.D., Professor of Urology, Johns Hopkins University, Baltimore. Cloth. Pp. 649, with 534 illustrations by William P. Didusch. Price, $10. Baltimore: Williams & Wilkins Company, 1937.

This book is a noteworthy contribution to the study and treatment of genital abnormalities. The first seven chapters are devoted to hermaphroditism in its various forms and aspects, and included are elaborate reports of eighteen cases minutely studied, in which the author operated. To the nineteen cases of human hermaphroditismus verus in the literature he adds a case in which the patient was subjected with success to surgical treatment. He discusses the adrenogenital syndrome in detail and describes two cases illustrating advances in operations on the adrenal. Other topics are: vaginal abnormalities in hermaphroditism; masculinization due to ovarian tumor; prostates in females; hypergenitalism and hypogenitalism; gynecomastia; hyperspadias and epispadias; exstrophy of the bladder; cryptorchidism; congenital valve obstruction in the prostatic urethra; abnormalities of the epididymis, vasa efferentia and seminal vesicles; atresia ani urethralis; the relation of the genital tract to the endocrine glands. The book has 574 instructive drawings by William P. Didusch of anatomic conditions and details of surgical operations. It is written clearly and reads easily. At all times the author reviews carefully the nature and the results of the abnormal developments for the treatment of which he has devised and practiced the different successful surgical procedures described in the detailed accounts of individual cases. But the book is of interest not only from the surgical point of view. It is a great storehouse of information to all students of genital abnormalities—embryologists, anatomists, pathologists, endocrinologists and clinicians in the broad sense. It will remain long a landmark in its field.

# Books Received

THE PATIENT AND THE WEATHER. William F. Petersen, M.D., with the assistance of Margaret E. Milliken, M.S. Volume 4, part 3. Organic Disease, Surgical Problems. Cloth. Price, $10. Pp. 651, with 482 graphs. Ann Arbor, Mich.: Edwards Brothers, Inc., 1938.

PRACTICAL BACTERIOLOGY, HEMATOLOGY AND ANIMAL PARASITOLOGY. E. R. Stitt, M.D., Sc.D., LL.D., Rear Admiral, Medical Corps, and Surgeon General, United States Navy (Rtd.); Paul W. Clough, M.D., Chief of the Diagnostic Clinic, Johns Hopkins Hospital, and Associate in Medicine, Johns Hopkins University; and Mildred C. Clough, M.D., Formerly Fellow in Bacteriology and Instructor in Medicine, Johns Hopkins University. Cloth. Price $7. Pp. 961, with 208 illustrations. Philadelphia: P. Blakiston's Son & Co., Inc., 1938.

A TEXTBOOK OF HEMATOLOGY. William Magner, M.D., D.P.H., Pathologist, St. Michael's Hospital, Toronto, and Lecturer in Pathology, University of Toronto. Cloth. Price, $4.50. Pp. 395, with 26 illustrations. Philadelphia: P. Blakiston's Son & Company, Inc., 1938.

ARBEITEN AUS DEM SERO-BAKTERIOLOGISCHEN INSTITUT DER UNIVERSITÄT HELSINKI. Volume 9 (1936-1937). Edited by Prof. Dr. Osw. Streng. Various pagination. Helsingfors, Finland, 1937.

MEDDELELSER FRA DR. F. G. GADES PATOLOGISK-ANATOMISKE LABORATORIUM I BERGEN. Various pagination. Bergen, Norway, 1938.

ARBEIDER FRA UNIVERSITETETS PATOLOGISK-ANATOMISKE INSTITUT VED RIKS-HOSPITALET FOR ÅRET 1937. Various pagination. Oslo, Norway, 1937.

THE TREATMENT OF CLINICAL AND LABORATORY DATA. AN INTRODUCTION TO STATISTICAL IDEAS AND METHODS FOR MEDICAL AND DENTAL WORKERS. Donald Mainland, M.B., Ch.B., D.Sc. (Edinburgh), Professor of Anatomy, Dalhousie University, Halifax, Nova Scotia, Canada. Cloth. Price, 15 shillings. Pp. 340, with 23 figures. Edinburgh: Oliver & Boyd, 1938.